EPIDEMIOLOGY OF 'EXTENDED SPECTRUM BETA LACTAMASE' PRODUCING ISOLATES OF *ESCHERICHIA COLI* AND *KLEBSIELLA* SPP AMONG BACTEREMIC ADULTS, ADMITTED TO A TERTIARY CARE TEACHING HOSPITAL

Epidemiology of 'Extended Spectrum Beta Lactamae'

producing isolates of *Escherichia coli* and *Klebsiella* spp

among bacteremic adults admitted to a tertiary care teaching hospital.

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF

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GENERAL MEDICINE EXAMINATION OF THE TAMILNADU

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C E R T I F I C A T E

This is to certify that the dissertation entitled **Epidemiology of 'Extended Spectrum Beta Lactamase' producing isolates of *Escherichia coli* and *Klebsiella* spp among bacteremic adults admitted to a tertiary care teaching hospital** *is* the bonafide original work of Dr. K. Paul Prabhakar Abhilash, towards the M.D. Branch-I (General Medicine) Degree Examination of the Tamil Nadu Dr. M.G.R University, Chennai, to be conducted in March, 2008.

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CERTIFICATE

This is to certify that the dissertation entitled **Epidemiology of 'Extended Spectrum Beta Lactamase' producing isolates of *Escherichia coli* and *Klebsiella* spp among bacteremic adults admitted to a tertiary care teaching hospital** *is* the bonafide original work of Dr. K. Paul Prabhakar Abhilash, towards the M.D. Branch-I (General Medicine) Degree Examination of the Tamil Nadu Dr. M.G.R University, Chennai, to be conducted in March, 2008.

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CONTENTS

INTRODUCTION

The advent of plasmid mediated ESBL production by *Klebsiella* and *E. coli* in the early 1980s signaled an emerging and evolving global problem with antibiotic resistance among Enterobacteriaceae. These organisms were susceptible to β-lactam antibiotics, but, with widespread use, have developed resistance through production of β-lactamases, which are the major defense mechanism of gram-negative bacteria against β-lactam antibiotics, and were first reported in the early 1980s.[1]

The presence of ESBLs carries tremendous clinical significance. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides), thus limiting the antibiotic options available for treatment of resistant strains .[2] Thus, it becomes imperative to quantify the problem, and reinforce guidelines promoting appropriate antibiotic use.

Since β-lactam antibiotics came into clinical use, β-lactamases have coevolved with them. As and when agents like cephamycins, cephalosporins with an oxyimino side chain, carbapenems, and aztreonam, that could break through the antimicrobial resistance were introduced, bacteria responded with a plethora of "new" β-lactamases —including extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC enzymes, and carbapenem-hydrolyzing β-lactamases (carbapenemases) — that, with variable success, can confer resistance to the latest β-lactam antibiotics.[3] Presumably the selective pressure by the use and overuse of these new introductions periodically for the treatment of patients, has allowed the proliferation and survival of new variants of

β-lactamase. Thus, ESBLs represent an impressive example of the ability of gram-negative bacteria to develop new antibiotic resistance mechanisms in the face of the introduction of new antimicrobial agents.

Antimicrobial resistance development is a function of bacterial genetic variability, such as the replication or generation rate in response to environmental stress (physical, nutrient availability etc), and selection pressure due to the use of similar antibiotics (class). The spreading antimicrobial resistance may be due to dispersion of resistant clones, or the genomes rapidly expanding its reservoirs or massive consumption of antimicrobials due to worldwide usage.

The prevalence of ESBL production among gram-negative bacilli varies widely and has been found to range from 3.3% to 86.6%, depending upon the clinical setting and the geographic region[43]In a study done in a tertiary care centre in India, ESBL was detected in in 80% of *Klebsiella spp*. and 68% among all gram negative bactria.[4] Risk factors for the occurrence of ESBL producing organisms have been studied in various settings and include an increased length of stay in the hospital, an increased length of stay in the intensive care unit, increased severity of illness emergency abdominal surgery, prior administration of an oxyimino-β-lactam antibiotic, and prior administration of any antibiotic.[5-8]

It must be admitted there are few national policies or guidelines governing rational antibiotic use. This study looks at the magnitude of this problem in a tertiary care setting and the role of antibiotic administration, in promoting the

development of resistant strains, and in being appropriate or otherwise, for containing such strains.

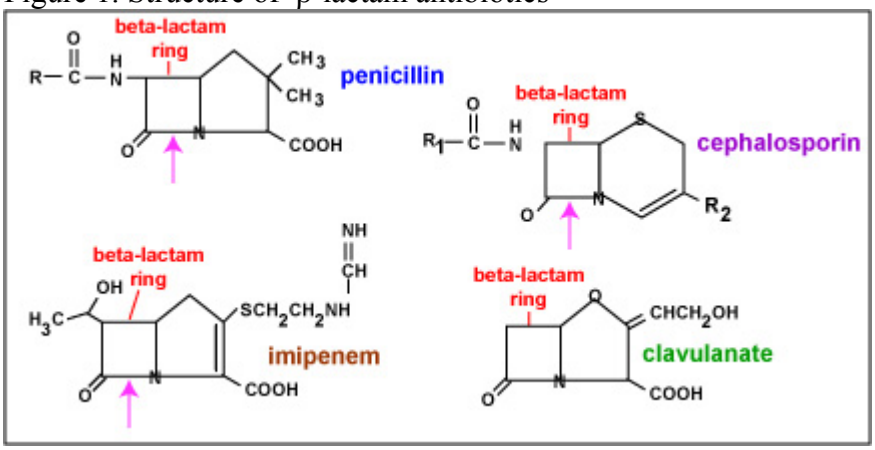
Previous studies have shown a significant effect of ESBL production on mortality. However, there are no prospective studies to evaluate the risk factors for mortality among patients with severe infections due to ESBL producing strains of *E. coli* and *Klebsiella.* This study was mainly done to assess the risk factors for ESBL production, antibiotic details and appropriateness and outcome in patients with the above infections.

OBJECTIVES OF THE STUDY

To determine

1. The prevalence of ESBL producing isolates of *E.coli* and *Klebsiella* among bacteremic adult hospitalized patients,
2. The rates of ESBL production among community acquired and nosocomial infections,
3. The risk factors for bacteremia due to ESBL producing *E. coli* and *Klebsiella* species.
4. The outcome of antibiotic treatment in bacteremia caused by *E. coli* and *Klebsiella* species in hospitalized adults.

Figure 1. Structure of β-lactam antibiotics



REVIEW OF LITERATURE

Overview of the β-lactam antibiotics

β-lactam antibiotics are among the most commonly prescribed drugs; they are grouped together based upon a shared structural feature, the β - lactam ring. β - lactam antibiotics include: Penicillins, Cephalosporins, Cephamycins, Carbapenems and Monobactams.[9] The basic structure of these antibiotics is shown in figure 1. "R" represents sites where different chemical side chains attach, depending on the particular antibiotic.

Mechanism of action[9]

β-lactam antibiotics inhibit the growth of sensitive bacteria by inactivating enzymes located in the bacterial cell membrane, which are involved in the third stage of cell wall synthesis. It is during this stage that linear strands of peptidoglycan are cross-linked into a fishnet-like polymer that surrounds the bacterial cell and confers osmotic stability in the hypertonic milieu of the infected patient. β-lactams inhibit not just a single enzyme involved in cell wall synthesis, but a family of related enzymes (four to eight in different bacteria), each involved in different aspects of cell wall synthesis. These enzymes can be detected by their covalent binding of radioactively-labeled penicillin (or other β-lactams) and hence have been called penicillin binding proteins (PBPs).[2] Different penicillin binding proteins appear to serve different functions

for the bacterial cell. As an example, PBP2 in Escherichia coli is important in maintaining the rod-like shape of the bacillus, while PBP3 is involved in septation during cell division.[10] Different β-lactam antibiotics may preferentially bind to and inhibit certain PBPs more than others. Thus, different agents may produce characteristic effects on bacterial morphology and have different efficacies in inhibiting bacterial growth or killing the organism.

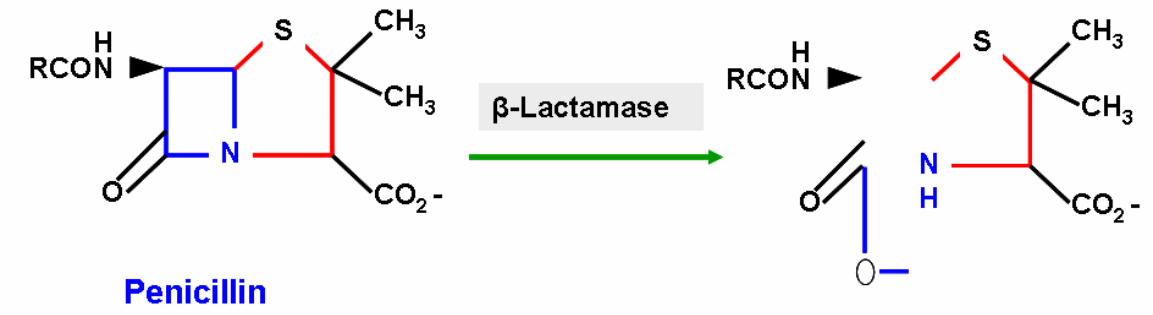
β-lactam antibiotics are generally bactericidal against organisms that they inhibit. The mechanism of bacterial cell killing is an indirect consequence of the inhibition of bacterial cell wall synthesis. Enzymes that mediate autolysis of peptidoglycan are normally present in the bacterial cell wall but are strictly regulated to allow breakdown of the peptidoglycan only at growing points. β-lactam inhibition of cell wall synthesis leads to activation of the autolytic system through a two component system, VncR/S, which initiates a cell death program.[11] Certain bacteria are deficient in these autolytic enzymes or have mutations in the regulatory genes; these strains show the phenomenon of "tolerance" to β-lactam antibiotics, that is, their growth is inhibited by the antibiotic but the bacteria are not killed.

Mechanisms of bacterial resistance[12,13]

Three general mechanisms of bacterial resistance to antibiotics, including the β-lactams, have been well characterized: decreased penetration to the target site; alteration of the target site; and inactivation of the antibiotic by a bacterial enzyme.

Decreased penetration to the target site: The outer membrane of Gram negative bacilli provides an efficient barrier to the penetration of beta-lactam antibiotics to their target

Figure 2. Mechanism of action of β-lactamases.



1. PBPs in the bacterial plasma membrane. β-lactams usually must pass through the hydrophilic porin protein channels in the outer membrane of Gram negative bacilli to reach the periplasmic space and plasma membrane. The permeability barrier of the outer membrane is a major factor in the resistance of *Pseudomonas aeruginosa* to many β-lactam antibiotics**.**

2. Alteration of the target site: The target sites for the β-lactams are the PBPs in the cytoplasmic membrane. Alterations in PBPs may influence their binding affinity for beta-lactam antibiotics and therefore the sensitivity of the altered bacterial cell to inhibition by these antibiotics. Such a mechanism is responsible for penicillin resistance in pneumococci[14], methicillin (oxacillin) resistance in staphylococci[15], and for bacteria with increasing intrinsic resistance to β-lactams, such as gonococci, enterococci, and *Haemophilus influenzae.*

3. Inactivation by a bacterial enzyme**:** Production of β-lactamase is the major mechanism of resistance to the β-lactam antibiotics in clinical isolates. Such bacterial enzymes may cleave predominantly penicillins (penicillinases), cephalosporins (cephalosporinases), or both (β-lactamases), shown in figure 2. Their production may be encoded within the bacterial chromosome (and hence be characteristic of an entire species) or the genes may be acquired on a plasmid or transposon (and hence be characteristic of an individual strain rather than the species). Bacteria may synthesize the β-lactamase constitutively (as for many plasmid-mediated enzymes) or synthesis may be inducible in the presence of antibiotic (as for many chromosomal enzymes). Inducible β-lactamases may not be reliably detected by initial susceptibility testing, particularly with the newer rapid methods.

Table 1. Key dates of evolution of ESBL

Year	Enzyme	Organism	Place
1944	Penicillinase	*S. aureus*	-
1963	TEM-1	*E. coli*	Athens
1974	SHV-1	*E. coli*	Switzerland
1978	OXA-10	*P. aeruginosa*	-
1982	SME-1	*S. marcescens*	London
1984	IMI-1	*E. cloacae*	California
1988	Metallo β-lactamase	*P. aeruginosa*	Japan
1989	Inhibitor-resistant penicillinase	*E. coli, K. pneumoniae*	France, Spain, Greece
1990	NMc A	*E. cloacae*	Paris
1991	OXA-11 OXA-14	*P. aeruginosa*	Turkey
1991	PER-1	*P. aeruginosa, S. typhimurium*	Turkey
1992	MEN-1	*E. coli, K. pneumoniae*	France
1994	TOHO-1	*E. coli*	Japan
1996	PER-2	*E. coli, K. pneumoniae,* *S. typhimurium, P. mirabilis*	Germany
1997	VEB-1	*E. coli*	Germany

Source: Chaudary et al. ESBL. An emerging threat to clinical therapeutics. IJMR 2004;22(2): 75 – 80.

Evolution and Dissemination of β-Lactamases [9]

Fifty years ago, the antibiotic era began with the discovery of penicillin. With in a few years of introduction of penicillin into clinical use, penicillinase producing *Staphylococcus aureus* started to proliferate in hospitals. To overcome this problem, penicillinase resistant penicillins came into picture. Shortly afterward, the broad spectrum penicillins and first generation cephalosporins were introduced. They remained a first line of defense against microbes for over 20 years, before resistance due to β-lactamases produced by gram negative bacilli became a serious problem.[2] To counter this threat, the pharmaceutical industry marketed six novel classes of β-lactam antibiotics (cephamycins, oxyimino cephalosporins, carbapenems, monobactams and clavam and penicillianic acid sulfone inhibitors) within a relatively short span of 7-8 years. Although, novel β-lactamases had emerged gradually after the introduction of new β- lactam agents, their number and variety accelerated at an alarming rate.[16] More than 170 β-lactamases have been recognized at present.

ESBLs are most likely to be found in *K. pneumoniae, K. oxytoca*, and *E. coli* but have been reported in Citrobacter, Enterobacter, Proteus, Salmonella, Serratia, and other genera of enteric organisms[17] and in such nonenteric organisms as *Acinetobacter baumannii* [18,19] and *P. aeruginosa*.[20]

The evolution of these enzymes over the years is shown in Table1.

Table 2. The Bush Jacoby Medeiros classification

Group	Enzyme Type	Inhibition by Clavulanate	Molecular Class	No. of Enzymes	Example
1	Cephalosporinase	No	C	53	*E. cloacae* P 99, MIR-1
2a	Penicillinase	Yes	A	20	*S. aureus, S. albus*
2b	Broad spectrum	Yes	A	16	TEM-1, SHV-1
2be	Extended spectrum	Yes	A	38	TEM-3, SHV-2, *K. oxytoca K1*
2br	Inhibitor resistant	Diminished	A	9	TEM-30, TRC-1
2c	Carbenicillinase	Yes	A	15	PSE-1, CARB-3, BRO-1
2d	Cloxacillinase	Yes	D or A	18	OXA-1, PSE-2, *Streptomyces cacaoi*
2e	Cephalosporinase	Yes	A	19	*Proteus vulgaris, Bacteroides fragilis Cep A*
2f	Carbapenemase	Yes	A	3	*E. cloacae* IMI-1, NMC-A
3	Metalloenzyme	No	B	15	*Xanthomonas maltophilia L1*
4	Pencillinase	No		7	*Pseudomonas cepacia*

Source: Chaudary et al. ESBL. An emerging threat to clinical therapeutics. IJMR 2004;22(2): 75 – 80.

Classification of Extended spectrum β-lactamases

Various classification schemes have been proposed by many researchers.[21] Classification of Sawai et al,[22] in 1968 was based on response to antisera and the Richmond and Sykes scheme in 1973 was on the basis of substrate profile. Extension of the Richmond and Sykes scheme by Sykes and Mathew in 1976, was based on differentiation by isoelectric focussing.[23]) In the scheme proposed by Mitsuhashi and Inoue in 1981,[24] the category "cefuroxime hydrolyzing β-lactamases" was added to "penicillinase and cephalosporinase" classification. The groupings proposed by Bush in 1989 was based on correlation of substrate and inhibitory properties with molecular structure.[21] However, the number and variety of enzymes have proliferated beyond the boundaries of the scheme. A more modern scheme based on molecular structure classification was proposed by Ambler,[25] includes only those enzymes that have been characterized. Recently a new classification scheme has been developed to integrate functional and molecular characteristics. The Bush-Jacoby-Medeiros scheme[2] classifies a total of 178 β-lactamases from naturally occurring bacterial isolates into four groups based on substrate and inhibitor profiles, this is shown in table 2. The enzymes can be classified on the basis of their primary structure into four molecular classes (A through D),[25] or on the basis of their substrate spectrum and responses to inhibitors into a larger number of functional groups. Class A and class C β-lactamases are the most common and have a serine residue at the active site, as do class D β-lactamases. Class B comprises the metallo-β-lactamases.

Important ESBL s :

TEM-Type ESBLs (Class A) :

Amino acid substitutions at many sites in TEM-1 β-lactamases can be created in the laboratory without loss of activity.[26] Those responsible for the ESBL phenotype change the configuration of the active site of the enzyme, allowing access to oxyimino-β-lactams.[27,28] Opening the active site to β-lactam substrates also typically enhances the susceptibility of the enzyme to β-lactamase inhibitors, such as clavulanic acid. Amino acid substitutions distinct from those leading to the ESBL phenotype can confer resistance to inhibitors, but the combination of inhibitor resistance and an extended spectrum of activity seems to be, with rare exceptions incompatible.[29] More than 130 TEM enzymes are currently recognized, and their variety provides a useful way to follow the spread of individual resistance genes. TEM-10, TEM-12, and TEM-26 are among the most common in North and South America.[7]

SHV-Type ESBLs (Class A) :

SHV-1 shares 68 percent of its amino acids with TEM-1 and has a similar overall structure.[30] As with TEM, SHV-type ESBLs have one or more amino acid substitutions around the active site. More than 50 varieties of SHV are currently recognized on the basis of unique combinations of amino acid replacements. SHV-type ESBLs currently predominate in surveys of resistant clinical isolates in Europe and America. [7,31] SHV-5 and SHV-12 are among the most common members of this family.

CTX-M–Type ESBLs (Class A) :

The most common group of ESBLs not belonging to the TEM or SHV families was termed CTX-M to highlight their greater activity against cefotaxime than against ceftazidime. More than 40 CTX-M enzymes are currently known. Belying their name, some hydrolyze ceftazidime more rapidly than they do cefotaxime. CTX-M-14, CTX-M-3, and CTX-M-2 are the most widespread of these.

Other Class A ESBLs:

Other Class A ESBLs are uncommon and have been found mainly in *Pseudomonas aeruginosa* and at a limited number of geographic sites: PER-1 in isolates in Turkey, France, and Italy; VEB-1 and VEB-2 in strains from Southeast Asia; and GES-1, GES-2, and IBC-2 in isolates from South Africa, France, and Greece. [20] PER-1 is also common in multi resistant *Acinetobacter* species in Korea and Turkey. [32] Some of these enzymes are found in Enterobacteriaceae as well, whereas other uncommon ESBLs (such as BES-1, IBC-1, SFO-1, and TLA-1) have been found only in Enterobacteriaceae.[33]

OXA-Type ESBLs (Class D) :

Twelve ESBLs derived from OXA-10, OXA-1, or OXA-2 by amino acid substitutions are currently known.[34] They have been found mainly in *P. aeruginosa* in specimens from Turkey and France. Most OXA-type ESBLs are relatively resistant to inhibition by clavulanic acid. Some confer resistance predominantly to ceftazidime, but OXA-17 confers greater resistance to cefotaxime and cefepime than it does resistance to ceftazidime.[35]

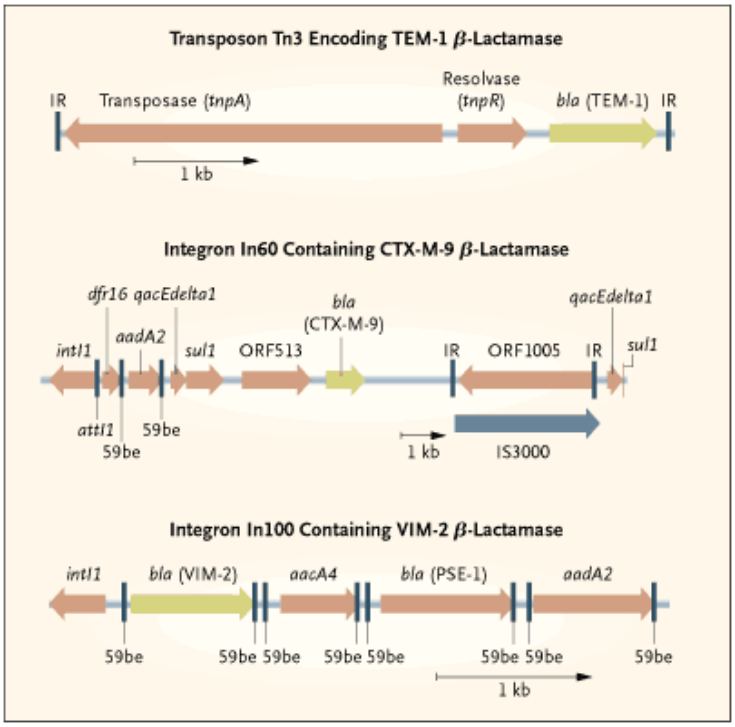
Plasmid-Mediated AmpC Enzymes (Class C) :

AmpC β-lactamases, usually inducible by β-lactams, are encoded by chromosomal genes in many gram-negative bacilli. Mutations that increase their expression are responsible for the ready emergence of broad-spectrum cephalosporin resistance in Enterobacter cloacae. [36] The AmpC enzyme in *E. coli* is poorly expressed and the AmpC gene is missing from the chromosome of *Klebsiella* and *Salmonella* species, but plasmid-mediated AmpC enzymes can give these organisms the same resistance profile as a β-lactam–resistant enterobacter isolate. More than 20 different AmpC β-lactamases have been found to be mediated by plasmids. Some, like the parental chromosomal enzymes, are accompanied by regulatory genes and are inducible, but most are not. Characteristically, AmpC β-lactamases provide resistance to cephamycins as well as to oxyimino-β-lactams and are resistant to inhibition by clavulanic acid.

Carbapenemases (Classes A, B, and D) :

Carbapenemases are a diverse group of enzymes. They are currently uncommon but are a source of considerable concern because they are active not only against oxyimino-cephalosporins and cephamycins but also against carbapenems [37].Plasmid-mediated IMP-type carbapenemases, 17 varieties of which are currently known, became established in Japan in the 1990s in both enteric gram-negative organisms and in pseudomonas and *acinetobacter* species. IMP enzymes spread slowly to other countries in the Far East, were reported from Europe in 1997, and have been found in Canada and Brazil. A second

Figure 3. Genetics of β-lactamases



IR denotes inverted repeat, bla 3-lactamase gene, dfr dihydrofolate reductase gene, qac gene conferring resistance to quaternary ammonium compounds, delta deletion derivative, intI site-specific integrase gene, aad aminoglycoside adenylyltransferase gene, sul dihydropteroate synthetase gene, ORF open-reading frame, attI recombination site, 59be 59-base element, aac aminoglycoside acetyltransferase gene, and IS insertion sequence.

growing family of carbapenemases, the VIM family, was reported from Italy in 1999 and now includes 10 members, which have a wide geographic distribution in Europe, South America, and the Far East and have been found in the United States . A few class A enzymes, notably the plasmid-mediated KPC enzymes, are effective carbapenemases as well. Finally, some OXA-type β-lactamases have carbapenemase activity, augmented in clinical isolates by additional resistance mechanisms, such as impermeability or efflux.

Genetics of β-Lactamases

Plasmids are responsible for the spread of most of the new β-lactamases, but the genes encoding these enzymes may also be located on the bacterial chromosome. The genes encoding some β-lactamases are carried by transposons.. Genes for many of the new β-lactamases are found in integrons, which often include genes conferring resistance to other antibiotics. For this reason, the new β-lactamases are usually produced by organisms that are resistant to multiple antimicrobial agents. Occasionally, the ESBL phenotype emerges in an organism isolated from a patient treated for multiple episodes of bacteremia, but much more often an ESBL-producing plasmid or strain disseminates to multiple patients, so that in hospital outbreaks one type of ESBL often predominates. Particular TEM-type ESBL varieties seem to have a fixed geographic distribution, whereas at least some SHV types have been found all over the world, suggesting that they have a multifocal origin. For example, TEM-3 is common in France and has been reported in a few other European countries but has not been reported in the United States, whereas SHV-5 and SHV-12 have been detected worldwide. The genes encoding the TEM-1 and TEM-2 β-lactamases are carried by transposons, as are the genes encoding

some TEM-type ESBLs .The gene encoding SHV-1 is found on the chromosome of most strains of *K. pneumoniae*. SHV genes also occur on transmissible plasmids; for example,

one has been found on a 7.5-kb block of DNA apparently captured from the *Klebsiella* chromosome. Genes encoding the remaining types of β-lactamase are often found incorporated into integrons but have their origin elsewhere. For example, the genes for CTX-M–type enzymes are found on the chromosome of Kluyvera, a genus of rarely pathogenic commensal organisms. Rather than evolving from a progenitor with a more limited spectrum of activity, the CTX-M group appears to have emerged in multiple places by plasmid acquisition of β-lactamase genes from such a widespread environmental reservoir. The genetic units encoding various β-lactamases are schematically depicted in figure 3. Diagrams of transposons and integrons encoding TEM-1, CTX-M-9, and VIM-2 -lactamases are shown. Integrons are also involved in the acquisition of AmpC-type β-lactamases by plasmids. Many of these plasmid-mediated enzymes can be related to chromosomal mpC enzymes of particular species: thus, ACC-1 is related to the enzyme produced by *Hafnia alvei*; ACT-1 and MIR-1 to enzymes of enterobacter species; some CMY enzymes as well as LAT-1 and LAT-3 to enzymes of citrobacter species; other CMY enzymes and the FOX and MOX families to enzymes of aeromonas species; and DHA-1 to the enzyme of *Morganella morganii*. Carbapenemases of the IMP and VIM families are also found within integrons , but the origin of their genes is not yet known.

Factors Influencing β-Lactamase Expression:

In addition to the vast number of enzymes, further complications arise because expression of resistance is affected by additional factors. The same enzyme may express different resistance phenotypes, depending on the bacterial host and the test conditions. For ESBLs of the TEM and SHV families, the expanded spectrum is

accompanied by a loss of intrinsic hydrolytic activity. This loss can be compensated for by an increase in gene dosage (through gene duplication or carriage on a multi copy plasmid) or the presence of a promoter with increased activity (through a mutation or insertion-sequence substitution). In some organisms (*Ps. aeruginosa* in particular), an active efflux system can reduce the intracellular accumulation of antibiotic and allow an enzyme with only limited hydrolytic capacity to inactivate the drug before it can reach its target; in other organisms, this effect is achieved by diminished expression of an outer-membrane porin required for β-lactam uptake. In *Klebsiella pneumoniae*, decreased expression of outer-membrane porins often accompanies ESBL production and may allow a TEM- or SHV-type ESBL to express resistance to cefepime or allow an AmpC β-lactamase to express resistance to Imipenem.

Escherichia coli [38]

The genus *Escherichia* is named after Theodor Escherich, who isolated the type species of the genus. They are gram-negative bacilli occurring singly or in pairs. *Escherichia coli* is facultatively anaerobic with both a fermentative and respiratory type of metabolism. They are either nonmotile or motile by peritrichous flagella. *E coli* is a major facultative inhabitant of the large intestine. *E. coli* can generally cause several

intestinal and extra-intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and Gram-negative pneumonia. Other miscellaneous infections that may be caused by *E coli* include septic arthritis, endophthalmitis, suppurative thyroiditis, sinusitis, osteomyelitis, endocarditis, or skin and soft tissue infections (especially in patients who are diabetic).

The enteric *E. coli* are divided on the basis of virulence properties into

1. Enterotoxigenic (ETEC, causative agent of diarrhea in humans, pigs, sheep, goats, cattle, dogs, and horses),
2. Enteropathogenic (EPEC, causative agent of diarrhea in humans, rabbits, dogs, cats and horses);
3. Enteroinvasive (EIEC, found only in humans), verotoxigenic (VTEC, found in pigs, cattle, dogs and cats);
4. Enterohaemorrhagic (EHEC, found in humans, cattle, and goats, attacking porcine strains that colonize the gut in a manner similar to human EPEC strains) and
5. Enteroaggregative *E. coli* (EAggEC, found only in humans).

Isolates from symptomatic infections of the urinary tract, bloodstream, cerebrospinal fluid, respiratory tract, and peritoneum (spontaneous bacterial peritonitis) are distinct from commensal and intestinal pathogenic strains of E. coli by virtue of their functionally similar virulence factor profiles and clonal background. It has recently been proposed that these extraintestinal strains of E. coli be termed ExPEC**.** Like commensal E. coli (but in contrast to intestinal pathogenic E. coli), ExPEC strains are often found in the normal intestinal flora and do not cause gastroenteritis in humans. Although acquisition of an ExPEC strain by the host is a prerequisite for ExPEC infection, it is not

the rate-limiting step, which instead is entry of a colonizing ExPEC strain from its site of colonization (e.g., the colon, vagina, or oropharynx) into a normally sterile extraintestinal site (e.g., the urinary tract, peritoneal cavity, or lungs). ExPEC strains have acquired genes encoding diverse extraintestinal virulence factors that enable the bacteria to cause infections outside the gastrointestinal tract in both normal and compromised hosts [39] .

E. coli is the most common enteric gram-negative species to cause extraintestinal infection in ambulatory, long-term-care, and hospital settings. [39]

Bacteremia caused by *E.coli*

E. coli bacteremia can arise from primary infection at any extraintestinal site. In addition, primary *E. coli* bacteremia can arise from percutaneous intravascular devices or can result from the increased intestinal mucosal permeability seen in neonates and in the settings of neutropenia and chemotherapy-induced mucositis, trauma, and burns. Roughly equal proportions of bacteremia cases originate in the community and in the hospital. *E. coli* and *Staphylococcus aureus* are the most common clinically significant blood isolates; *E. coli*, which is isolated in 17 to 37% of cases, is the gram-negative bacillus most often isolated from the blood in the ambulatory setting as well as in most long-term-care and hospital settings. Isolation of *E. coli* from the blood is almost always clinically significant and typically is accompanied by the sepsis syndrome, severe sepsis (sepsis-induced dysfunction of at least one organ or system), or septic shock, manifesting with hypotension and fever (in some cases, with hypothermia rather than fever). It may be complicated by uremia, hepatic failure, acute respiratory distress syndrome, stupor or coma, and death. Non–life-threatening bacteremia may manifest as a

sudden onset of fever and chills, tachycardia, tachypnea, and mental confusion. The urinary tract is the most common source of *E. coli* bacteremia, accounting for two-thirds of episodes[40]. Bacteremia from a urinary tract source is particularly common with pyelonephritis, urinary tract obstruction, or instrumentation in the presence of infected urine. The abdomen is the second most common source, accounting for 25% of episodes. Although obstructive biliary tract disease (stones, tumor) and overt disruption of bowel are responsible for many of these cases, some abdominal sources (e.g., abscesses) are remarkably silent clinically and require identification via imaging studies (e.g., computed tomography). Soft tissue, bone, and pulmonary infections are the next most common sources for bacteremia.

Klebsiella [38]

The genus *Klebsiella* belongs to the tribe Klebsiellae, a member of the family Enterobacteriaceae. The organisms are named after Edwin Klebs, a 19th century German microbiologist. *Klebsiellae* are nonmotile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms.

Members of the *Klebsiella* genus express 2 types of antigens on their cell surface. The first is a lipopolysaccharide(O antigen); the other is a capsular polysaccharide(K antigen). Both of these antigens contribute to pathogenicity. About 77 K antigens and 9 O antigens exist [41]. The structural variability of these antigens forms the

basis for classification into various serotypes; the virulence of all serotypes appears to be similar.

The genus was originally divided into 3 main species based on biochemical reactions. Today, 7 species with demonstrated similarities in DNA homology are known. These are

(1) *Klebsiella pneumoniae,*

(2) *Klebsiella ozaenae,*

(3) *Klebsiella rhinoscleromatis,*

(4) *Klebsiella oxytoca,*

(5) *Klebsiella planticola,*

(6) *Klebsiella terrigena,* and

(7) *Klebsiella ornithinolytica*.

K pneumoniae is the most medically important species of the group. *K oxytoca* and *K rhinoscleromatis* have also been demonstrated in human clinical specimens. In recent years, *Klebsiellae* have become important pathogens in nosocomial infections.

Klebsiellae are ubiquitous in nature. Carriage rates vary with different studies. *Klebsiellae* may be regarded as normal flora in many parts of the colon and intestinal tract and in the biliary tract. Oropharyngeal carriage has been associated with endotracheal intubation, impaired host defenses, and antimicrobial use. In healthy humans, *K. pneumoniae* colonization rates range from 5 to 35% in the colon and from 1 to 5% in the oropharynx; the skin is usually colonized only transiently. They may also

colonize sterile wounds and urine. Organisms can spread rapidly, often leading to nosocomial outbreaks.

The spectrum of clinical syndromes includes pneumonia, bacteremia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, and meningitis. Sepsis and septic shock may follow entry of organisms into the blood from a focal source. The presence of invasive devices,

contamination of respiratory support equipment, use of urinary catheters, and use of antibiotics are factors that increase the likelihood of nosocomial infection with Klebsiella species.

Bacteremia caused by *Klebsiella pneumoniae*

Klebsiella infection at any site can result in bacteremia. Infections of the urinary tract, respiratory tract, and abdomen each account for 15 to 30% of *Klebsiella* bacteremias. Intravascular device–related infection is another important source (5 to 15%). Surgical site infection and other miscellaneous infections account for the rest. *Klebsiella* is one of the agents that causes sepsis neonatorum and bacteremia with fever and neutropenia. Like enteric GNB in general, *Klebsiella* rarely causes endocarditis or endovascular infection.

Prevalence studies

Indian data:

In a dissertation by Mathai D, in our centre,[42] among 672 patients with blood stream , skin and soft tissue and closed space infections, caused by Gram-negative group study pathogens, 37.9% was community acquired and 28.8% was nosocomial. ESBL rate among community acquired - *E.coli* was 26.2%, 25% in *Klebsiella* spp., and 32.2% in *Enterobacter* spp. In the nosocomial group 72.8% was due to ESBL *E.coli*, 26.4% *Klebsiella* spp and 25.8% *Enterobacter* spp. Overall, among the Gram negative group a significant difference in the frequency of nosocomial occurrence of infection caused by ESBL *E.coli* was seen when compared to non ESBL-*E.coli.* The total number

of deaths (n=36) due to Gram- negative infections was 5.11% with 75% of them caused by ESBLs. Mortality due to ESBL causing blood stream infections was 47.2% and the remainder occurring among those with skin, soft tissue and closed space infections.. Early death occurred among those infected with ESBL *E.coli*. This significant difference was not seen among the infections caused by *Klebsiella* spp.

In a study of 678 Gram negative bacteria from various clinical samples obtained from indoor patients admitted to the All India Institute of Medical Sciences, New Delhi during March to June 2001, 458 (68%) were found to be ESBL producers. Among the bacterial species, ESBL production was most common in *Klebsiella spp*. (80%). The proportion of ESBL positive isolates was highest from intensive care units (79%), followed by Medical Oncology (75%), Medical (54%) and Surgical wards (50%).[4]

In a study done at the Departments of Microbiology, King George's Medical College, Lucknow, [43] ESBL was detected in 86.6% of *Klebsiella* spp., 73.4% of *Enterobacter* spp. and 63.6% of *Escherichia coli* strains. It was also observed that 74.4-80.9% of these ESBL producers were resistant to cefotaxime and 47.6-59.5% were resistant to ceftazidime in routine susceptibility testing. Some ESBL producers (36.3-61.5%) were found to be susceptible to either or both cephalosporins used in this study. [43]

In a study done among septicaemic neonates in neonatal intensive care units (NICU) at Post Graduate Department of Microbiology, King George's Medical University, Lucknow, India,[44] a total of 100 clinical isolates of *Klebsiella spp* isolated from 2995 blood samples of suspected cases of neonatal septicaemia were studied.Antimicrobial susceptibility was determined by Kirby- Bauer's disc diffusion method. Resistance pattern of ESBL producers and non-ESBL producers was compared. 58% of *Klebsiella* isolates were positive for ESBL production. Almost all the isolates were sensitive to imipenem and meropenem. Drug resistance was found to be significantly more common in ESBL producing isolates than in non-ESBL producers.[44]

In a study done at National Institute of Communicable Diseases, New Delhi in 2003, [45]51 *K. pneumoniae* isolates were confirmed from samples from pus, wound, pleural fluid, urine and tracheal aspirate of 395 patients attending respiratory, urology and burns wards. Antimicrobial susceptibility was carried out by Kirby Bauer's disc diffusion technique using NCCLS criteria. A screening of ESBL production was done by Double-disc synergy test (DDST) and using E-test ESBL strips. The frequency of resistance among *K. pneumoniae* for the cephalosporins (cefoxitin, cefuroxime,

cefotaxime, ceftazidime, and cefepime) and non-cephalosporins (aztreonam, piperacillin, chloramphenicol and trimethoprim-sulfamethoxazole) were in the range of 39.2-88.0% and 51.0-90.2% respectively. A total of 36 (70.6%) of the 51 isolates were could be identified as ESBL producers.

Data from other countries

Despite worldwide use of β-lactam antibiotics, the distribution of the enzymes responsible for resistance to oxyimino-cephalosporins and carbapenems is far from uniform. Some hospitals in the United States seem to have no ESBLs, whereas in other hospitals as many as 40 percent of *K. pneumoniae* isolates have been reported to be

ceftazidime-resistant as a result of ESBL production. [46,47] their prevalence is higher in isolates from intensive care units than in isolates from other hospital sites.

In a study by Winokur et al, [48] of more than 4700 *K. pneumoniae* isolates, the percentage expressing an ESBL phenotype was highest in isolates from Latin America (45.4 percent), the Western Pacific (24.6 percent), and Europe (22.6 percent) and lowest in strains from the United States (7.6 percent) and Canada (4.9 percent). In more than 13,000 isolates *of E. coli,* the percentages expressing the ESBL phenotype were as follows: in Latin America, 8.5 percent; in the Western Pacific, 7.9 percent; in Europe, 5.3 percent; in the United States, 3.3 percent; and in Canada, 4.2 percent.[48]

In another study from the United States, ceftazidime resistance was present in 9.6 percent of *K. pneumoniae* isolates from intensive care units and 6.6 percent of isolates from other hospital locations.[49]A resistant strain or plasmid may cause problems in several hospitals locally or involve a large geographic area.[50,51] Community

clinics and nursing homes have also been identified as potential reservoirs for ESBL-producing *K. pneumoniae* and *E. coli*.[52,53] In 1989, non typhoid *salmonella* strains producing CTX-M-2 began to spread among neonatal units in Argentina and to neighboring South American countries, and by 2002 this enzyme was present in about 75 percent of ESBL-producing Enterobacteriaceae in Buenos Aires.[54] CTX-M enzymes, which are also common in Japan, China, Korea, Taiwan, Vietnam, and India, have bee Kingdom[55] and have been reported in Eastern Europe, Germany, France, and Spain and recently in the United States.[56] It is estimated that in the United States, 3 to 4 percent of clinical *K. pneumoniae* and *K. oxytoca* isolates carry plasmid-mediated AmpC enzymes. One particular plasmid-mediated AmpC enzyme, CMY-2, has been responsible for increasing resistance to ceftriaxone and other oxyimino-β-lactams in salmonella isolates from the United States. In Japan, IMP-type carbapenemases, first detected in *Serratia marcescens* and *P. aeruginosa*, have spread to other gram-negative bacilli,[57] but the prevalence of this resistance mechanism is surprisingly low: 1.3 percent in *P. aeruginosa* and less than 0.5 percent in *E. coli* and *K. pneumoniae*[47,58,59] Considering the broad resistance to β-lactam antibiotics that is conferred by carbapenemases and considering their presence in Japan for more than a decade, their limited occurrence is reassuring, considering the potential for future spread. Worldwide, 99.9 percent of Enterobacteriaceae remain susceptible to carbapenems.[60] Carbapenemases can, however, be associated with lethal infections. In Greece and Italy, outbreaks due to carbapenem-resistant *P. aeruginosa* producing VIM-1 carbapenemase were identified in separate hospitals and associated with a high mortality rate. [47,61,62] In Brazil, a strain of *A. baumannii* resistant to imipenem and meropenem due to an OXA-type carbapenemase

infected eight patients in two hospitals; five of the patients died, despite therapy with multiple antibiotics, including polymyxin B.[47,63] *K. pneumoniae* strains with reduced susceptibility to carbapenems due to KPC-2 or KPC-3 have been recently reported in several hospitals in New York City.[64]

Risk Factors for Infection:

Reported risks, many of which are linked, include an increased length of stay in the hospital,[5,65,66] an increased of stay in the intensive care unit,[67,68] increased severity of illness,[5,6,69] the use of a central venous or arterial catheter,[5,67,67,67,68,70] the use of a urinary catheter,[67,6,68,69 70] ventilatory assistance,[68,6,71] hemodialysis,[72] emergency abdominal surgery,[68] the use of a gastrostomy or jejunostomy tube,[5] gut colonization,[67] prior administration of an oxyimino-β-lactam antibiotic,[5,6,8,73,74,89] and prior administration of any antibiotic .[5,70,75]

Problems in Detection [9]

Identifying organisms that are ESBL producers is a major challenge for the clinical microbiology laboratory. Due to the variable affinity of these enzymes for different substrates and inoculum effect, some ESBL isolates may appear susceptible to a third generation cephalosporin in vitro. However, treatment of infections due to an ESBL producing organism with third generation cephalosporins may result in clinical failure if infection is outside the urinary tract. [1] Cefpodoxime and ceftazidime have been proposed as indicators of ESBL production as compared to cefotaxime and ceftriaxone. Hence, a

centre where only cefotaxime and ceftriaxone are routinely tested, may have difficulty in detecting ESBLs.[1]

As, these enzymes can be induced by certain antibiotics, amino acids or body fluids, organisms possessing genes for inducible β-lactamases show false susceptibility if tested in the uninduced state. [76]

For ESBL producing bacteria there is a dramatic rise of the minimum inhibitory concentration (MIC) for extended spectrum cephalosporins when the inoculum is increased beyond that used in routine susceptibility. Some isolates test susceptible at the standard inoculum of 10^5 CFU/mL but are resistant at an inoculum of 10^7 CFU/mL. Therefore, they may be reported as false sensitive if tested by routine methods. [77]

Sensitivity breakpoints of MIC as designated by the Clinical and Laboratory Standards Institute(CLSI),[78] recommend screening *Klebsiella* spp. and *E. coli* isolates with a MIC greater than or equal to 2 mg/mL against cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone, as potential ESBL producers. Two indicators of ESBLs are an 8 fold reduction in MIC in the presence of clavulanic acid when using the broth dilution and the potentiation of the inhibitor zone by clavulanic acid (>5 mm increase in diameter of inhibition zone) when using disc diffusion method. These methods, though useful, may not detect those ESBLs that are poorly inhibited by β-lactamase inhibitors.[1]

Two recent studies evaluated the ability of clinical laboratories to detect and report the presence of ESBLs. A survey in Connecticut [1] found that 21% of laboratories fail to detect ESBL producing isolates. A proficiency testing project for

clinical laboratories participating in the National Nosocomial Infections Surveillance System [1] indicated that as many as 58% laboratories failed to detect and report ESBL isolates correctly. These data suggest that improvements in the ability of clinical laboratories to detect ESBL are needed.[1]

Methods of Detection

Several tests have been developed to confirm the presence of ESBLs.

Double disc synergy test [79]

In this test, discs of third generation cephalosporins and amoxicillin-clavulanic acid are kept 30 mm apart from center to center on inoculated Mueller-Hinton Agar (MHA). A clear extension of the edge of the inhibition zone of cephalosporin towards the amoxicillin-clavulanic acid disc is interpreted as positive for ESBL production.

Three dimensional test[80]

This test provides the advantage of simultaneous determination of the antibiotic susceptibility and the β-lactamase substrate profile. Inoculum produced in this method contains between 10^9 and 10^{10} CFU/mL of cells that actively produce β-lactamase. Two types of inocula are prepared, a disc diffusion test inoculum (optical density equal to that of 0.5 McFarland standard) and a three dimensional inoculum (contain between 10^9 and 10^{10} CFU/ml). Plate is inoculated by disc diffusion procedure. A circular slit is cut on the agar 4mm inside the position at which the antibiotic discs are placed. Conventional (two dimensional) disc diffusion susceptibility test results are measured according to the recommendations of the Clinical Laboratory Standard Institute

(CLSI).[78] Distortion or discontinuity in the circular inhibition zone is interpreted as positive for ESBL production.

Inhibitor potentiated disc diffusion test [81]

Cephalosporin disc is placed on clavulanate containing and with out clavulanate containing MHA plates. More than10 mm increase in the zone of inhibition on the clavulanate containing MHA plate indicates ESBL production.

Disk approximation test [76]

Cefoxitin (inducer) disc is placed at a distance of 2.5 cm from cephalosporin disc. Production of inducible β-lactamase is indicated by flattening of the zone of inhibition of the cephalosporin disc towards inducer disc by >1 mm.

MIC reduction test [1]

An 8 fold reduction in the MIC of cephalosporin in the presence of clavulanic acid indicates production of ESBL.

Vitek ESBL Test [82]

Four wells containing cards are inoculated. A predetermined reduction in growth of cephalosporin well containing clavulanic acid; when compared with the level of growth in well with cephalosporin alone indicates presence of ESBL.

E-Test[83]

The E test ESBL strip carries two gradients, on the one end, ceftazidime and on the opposite end ceftazidime plus clavulanic acid. MIC is interpreted as the point of intersection of the inhibition ellipse with the E test strip edge. Ratio of ceftazidime

MIC and ceftazidime clavulanic acid MIC equal to or greater than 8 indicates the presence of ESBL.

Disk Diffusion Susceptibility Testing (Kirby-Bauer Method):[84]

For the Disk Diffusion Test, a carrier (paper disk) impregnated with a known amount of antibiotic is placed on the surface of a solid medium, which has previously been inoculated with a bacterial suspension of the pathogen to be tested. The antibiotic diffuses from the carrier into the medium, producing a concentration gradient. Bacterial growth in the vicinity of the carrier only occurs when the concentration diffusing from the carrier is no longer sufficient to inhibit bacterial replication, or when the pathogen is resistant to the antibiotic in question. If the concentrations are sufficient to achieve inhibition, a circular region of no bacterial growth develops. This is called the zone of inhibition, figure 4 and the limits are mentioned in table 3.

The Clinical and Laboratory Standards Institute (CLSI) has developed broth microdilution and disk diffusion screening tests using selected antimicrobial agents Each *Klebsiella pneumoniae*, *K. oxytoca*, or *Escherichia coli* isolate should be considered a potential ESBL-producer based on disc diameter and MIC parameters, shown in table 4.

Figure 4. Disk diffusion test

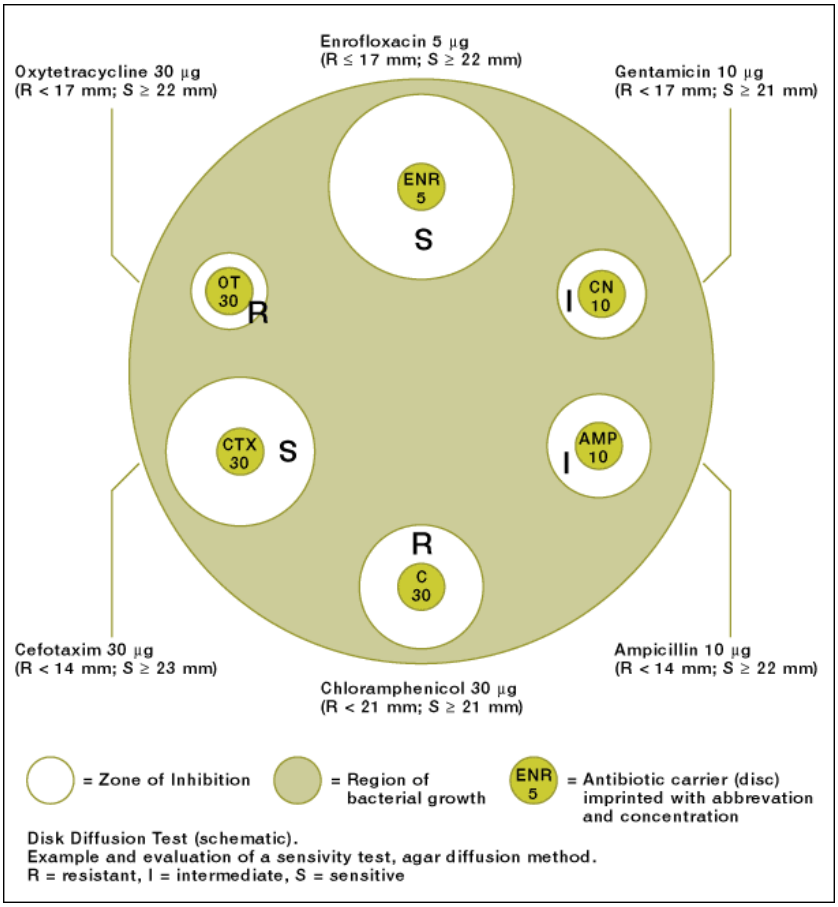


Table3. Defined limits for zone of inhibition

Judgement	**Inhibition zone diameter(mm)**	**MIC (microgram/ml)**
Sensitive	> 21	<0.5
Moderately sensitive	18 -21	1
Resistant	< 17	>2

Table 4. MIC and Inhibition Zone Criteria for the Detection of ESBLs in *K. pneumoniae* and *E.coli**

Antibiotic	**Zone diameter for isolates in mm**		**MIC in mg/ L**	
	Susceptible	**ESBL producing**	**Susceptible**	**ESBL producing**
Aztreonam 30µg	≥22	≤ 27	≤8	≥2
Cefotaxime 30µg	≥23	≤27	≤8	≥2
Cefpodoxime 30µg	≥ 21	≤ 22	≤8	≥2
Ceftazidime 30µg	≥18	≤ 22	≤8	≥2
Ceftriaxone 30µg	≥21	≤ 25	≤8	≥2

*adapted from NCCLS document M100-S8[8]

The sensitivity of screening for ESBLs in enteric organisms can vary depending on which antimicrobial agents are tested. [78]The use of more than one of the five antimicrobial agents suggested for screening will improve the sensitivity of detection. Cefpodoxime and ceftazidime show the highest sensitivity for ESBL detection.[78]

Confirmation of ESBL production:

CLSI recommends performing phenotypic confirmation of potential ESBL-producing isolates of *K. pneumoniae, K. oxytoca, o*r *E. coli* by testing both cefotaxime and ceftazidime, alone and in combination with clavulanic acid. Testing can be performed by the double disk diffusion test.

Disk Approximation or Double Disk Method : [85]

The disk approximation method uses either multiple target disks, or a single cefpodoxime disk, and a clavulanic acid disk. Disk content and the disk placement must follow a validated method.[86]

A Mueller-Hinton agar plate is inoculated with a suspension made from an overnight blood agar culture of the test strain as recommended for a standard disk diffusion susceptibility test. Disks containing the standard 30 µg of ceftazidime, or ceftriaxone, or aztreonam, or 10 µg of cefpodoxime are placed 15 mm apart (edge to edge) and from an amoxicillin-clavulanic acid disk containing 10 µg of the latter compound. The disk edge-to-edge distance recommended here is that reported7 as having greater sensitivity than the previous distance of 20 to 30 mm.

Following incubation for 16-20 hours at 35o C, any enhancement of the zone of inhibition between a β-lactam disk and that containing the β-lactamase inhibitor is indicative of the presence of an ESBL.

Precise placement of the disks, correct storage of the clavulanate-containing disks, and performance of appropriate control tests are critical to the sensitivity of the disk approximation method.[87]

This method seems to work well for ESBL-producing isolates of *K. pneumoniae* and *E. coli* but not with *Enterobacter* or *Morganella* isolates. *K. pneumoniae*

ATCC 700603 (positive control) and *E. coli* ATCC 25922 (negative control) are used for quality control of ESBL tests. The phenotypic confirmatory test does not detect all ESBLs. Some organisms with ESBLs contain other β-lactamases that can mask ESBL production in the phenotypic test, resulting in a false-negative test. These β-lactamases include AmpCs and inhibitor-resistant TEMs (IRTs). Hyper-production of TEM and/or SHV β-lactamases in organisms with ESBLs also may cause false-negative phenotypic confirmatory test results. Currently, detection of organisms with multiple β-lactamases that may interfere with the phenotypic confirmatory test can only be accomplished using isoelectric focusing and DNA sequencing,

Clinical and Laboratory Standards Institute guidelines 2006 :[78,88]

Strains of Klebsiella spp, *E. coli* and *Proteus mirabilis* that produce ESBL may be clinically resistant to therapy with penicillins, cephalosporins or aztreonam despite apparent invitro susceptibility to some of these agents, table 5. Some of these strains will show zones of inhibition below the normal susceptible population, but above the standard break points for certain extended spectrum cephalosporins or Aztreonam; such strains may be screened for potential ESBL production by using the screening break

points as recommended by CLSI .Other strains may test intermediate or resistant by standard breakpoints to one or more of these agents. In all strains with ESBL, the zone diameters for one or more of the extended spectrum cephalosporins should increase in the presence of clavulanic acid as described in the phenotypic confirmation test. For all confirmed ESBL producing strains, the test interpretation should be reported as resistant for all penicillins, cephalosporins and aztreonam.

Reporting of results [78]

1. **Susceptible (S)**

The "susceptible" category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection.

2. **Intermediate (I)**

The "intermediate" category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g., quinolones and β-lactams in urine) or when a higher than normal dosage of a drug can be used (e.g., β-lactams). This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

3. Resistant (R)

The "resistant" category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate zone diameters that fall in the range where specific microbial resistance

mechanisms (e.g., β-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

Antibiotic susceptibility

There is an alarming rise in ESBL production among *Klebsiella* and *E coli* strains and high rate of resistance to a wide range of cephalosporin and non-cephalosporin group of antimicrobials. A study was done to evaluate the ESBL production and in-vitro susceptibility of *K. pneumoniae* isolates from the National Institute of Communicable Diseases in New Delhi in 2004.[45] The bacterial isolates collected during 2003 included 51 *K. pneumoniae* biochemically confirmed isolates from 395 patients admitted in various wards of a major hospital in New Delhi. The isolates were from pus, wound, pleural fluid, urine and tracheal aspirate of patients attending respiratory, urology and burns wards. The frequency of resistance among *K. pneumoniae* for the cephalosporins (cefoxitin, cefuroxime, cefotaxime, ceftazidime, and cefepime) and non-cephalosporins (aztreonam, piperacillin, chloramphenicol and trimethoprim-sulfamethoxazole) were in the range of 39.2-88.0% and 51.0-90.2% respectively. 14 different antimicrobial resistance profiles were recognized ranging from resistance to only four (n=6, 11.7%) to as many as ten (n=9, 17.7%). (70.6%) 36/51 of the strains produced ESBL that correlates with the high frequency of multi-drug resistant *K. pneumoniae*.

In a multicentric study, done in south India by Mathai D ,[42] at CMCH and other sentry centers, Gram-negative study pathogens including MDR (resistance to ≥

three classes) strains showed excellent susceptibility to imipenem and meropenem (>95%). All ESBLs were MDR with high resistance rates (>90%) to β-lactam- β-lactamase inhibitor combination, fourth generation cephalosporins, quinolones and aminoglycosides. Among non ESBLs high susceptibility (>90%) to β-lactam- β-lactamase inhibitor combination, fourth generation cephalosporins, quinolones and aminoglycosides was observed. The antibiogram of ESBL producing Gram negative study pathogen among ICU and non ICU settings seen were similar. There was no difference in the susceptibility pattern of the community and hospital associated Gram-negatives.

Treatment

Of all the available β-lactams, carbapenems are the most effective and reliable, as they are highly resistant to the hydrolytic activity of all ESBL enzymes, due to the trans-6 hydroxy ethyl group.[1] Meropenem is the most active with MICs generally lower than those of imipenem (0.03-0.12mg/mL Vs 0.06-0.5mg/mL). Several new carbapenems, ertapenem and faropenem are being studied in the various phases of clinical trials.[1] Some infections due to organisms testing resistant to ceftazidime but

susceptible to cefotaxime or ceftriaxone, have responded to treatment with these alternate cephalosporins. However, MICs of these agents rise dramatically as the inoculum is increased.[34]

A few β-lactams, 7α methoxy cephalosporins such as cefoxitin,

cefmetazole, cefotetan and latamoxef are often effective in the treatment of infections caused by enzyme producing bacteria. However, cephamycins should be used with caution because of the relative ease with which clinical strains decrease expression of outer membrane proteins.[89]

Although ESBL activity is inhibited by clavulanic acid, the only infections that may be treated safely with β- lactam/β-lactamase inhibitor combination are those involving the urinary tract. In this instance, β- lactamase inhibitor concentration is high enough to counteract the hydrolytic activity of ESBLs. [16]

By inhibiting ESBL, β-lactamase inhibitors appear to impair the emergence and spread of *Klebsiella* carrying resistance plasmids. Furthermore, administration of inhibitors may exert in vitro pressure on ESBL, thereby facilitating their reverse mutation to less harmful enzymes.[71] Clavulanic acid appears more efficient than sulbactam, it takes about eight times more sulbactam to obtain a protection similar to that given by clavulanic acid [47].Non β-lactam antimicrobial agents (aminoglycosides, fluoroquinolones) may be beneficial, however, co resistance rates against these agents are frequent.[1]

Outbreak Control

In outbreak situations, successful control has usually involved both restriction of the use of oxyimino-β-lactams and the institution of barrier precautions (hand washing, gloves, and gowns) for patients with infection or colonization..[6,90,91]

Successful control with the use of strict isolation procedures without limitations on antibiotic use has also been reported.[92] Substitution of imipenem,[91] piperacillin–tazobactam,[93] or cefepime–amikacin[94] as the antibiotic of choice for empirical therapy has been followed by decreased isolation of ESBL-producing organisms.

Antibiotic substitutions can, however, have unintended consequences. In an outbreak of infection with *K. pneumoniae* resistant to other β-lactam antibiotics, increased use of imipenem was followed by the emergence of imipenem-resistant *K. pneumoniae* that produced an AmpC enzyme (ACT-1) and was missing an outer-membrane porin [64,95]. At the same hospital, increased use of imipenem also led to the emergence of imipenem-resistant *A. baumannii*. [96]

A report from Pondicherry Institute of Medical Sciences, Kalapet, Pondicherry in 2004[97] describes an outbreak of ESBL positive *K.pneumoniae* in a neonatal intensive care unit, which occurred over a period of 20 days resulting in death of 8 out of 10 neonates affected. The outbreak started with the isolation of *K. pneumoniae* from a preterm baby with severe respiratory distress and within a period of three weeks ten neonates developed *K.pneumoniae* septicaemia. Isolation of the septicaemic neonates was done to control spread of *K.pneumoniae* in the neonatal intensive care unit. Subsequent screening of the environment yielded *K.pneumoniae* from breast pump,

breast milk storage bottle and refrigerator. Antibiotic susceptibility testing of all clinical and environmental isolates showed a similar antibiogram. The breast pump and the milk storage bottle were identified as the most probable source responsible for the outbreak. Sterilization of breast pump and storage bottles subsequently controlled the outbreak.

Therefore institution of barrier precautions (hand washing, gloves, and gowns) for patients with infection or colonization is essential in controlling an outbreak of an ESBL producing organism.

Scoring systems

PITT s bacteremia score :[36]

This is a previously validated scoring system to assess the severity of the illness for prognostication. Severity of illness was assessed by this grading system that evaluated mental status (Disoriented -1, Stupor -2, Coma – 4 points); Fever (>= 37.6º C and < 40 º C -1 point ; >=40 º C -2 points); hypotension (drop in systole 20 mm Hg or diastolic > 10 mm Hg or on intravenous pressor agents -2 points);Mechanical ventilation -2 points and cardiac arrest –4 points. Patients accumulating 4 or more points were defined as severely ill. This grading system has previously been shown to be highly predictive of outcome in previous studies of *Klebsiella* and *Pseudomonas* bacteremia.[7, 36,98,99] The scoring system is appended, appendix 1.

SAPS II score :[100-102]

This is a standard scoring system used commonly in the ICU s of France, where it was first validated. SAPS II includes 15 variables including 12 physiology variables. SAPS II includes 15 variables including 12 physiology variables using which, the predicted mortality could be calculated.

The variables are as follows:

Age, type of admission (scheduled surgical , unscheduled surgical, or medical), underlying disease condition (AIDS, metastatic cancer, and hematologic malignancy), Glasgow Coma Scale(GCS), systolic blood pressure(SBP), heart rate, temperature, PaO_2/ FiO_2 on ventilatory support, urine output, serum urea/ blood urea nitrogen (BUN), WBC count, serum potassium, sodium, bicarbonate and bilirubin.

MATERIALS AND METHODS

Study Setting:

The study was conducted in Christian Medical College Hospital, Vellore, a 2500 bedded academic medical center in South India with an average of 1815 inpatients and approximately 3500 out- patient visits every day.

Study design:

This was a prospective cohort study.

Subjects:

The study recruited all sequentially encountered patients older than 16 years with *Klebsiella* or *E. coli* bacteremia who were admitted in various wards and Intensive care units of this hospital during the study period. We recruited a total of 131 patients over a 4 month period.

Inclusion criteria:

1. Isolation of *E. coli* or *Klebsiella* from blood culture samples and
2. Willingness to participate in the study.

Exclusion criteria:

1. Patients aged less than 16 years,
2. Outpatients ,
3. Bacteremias of polymicrobial etiology,
4. Patients unwilling for inclusion in the study.

Methodology:

A written informed consent was taken from either the patient, or from the legal guardian in those with altered mental status, prior to enrollment. All patients with *E coli* and *Klebsiella* bacteremia were evaluated within 48 hours of detection of a positive blood culture. Bacteremias of polymicrobial etiology were not included.

Blood culture was taken at the onset of fever by venupuncture from a peripheral vein after adequate preparation of the skin with povidone iodine (Betadine). A minimum of 5 ml of blood was inoculated into a blood culture bottle (BacT/ALERT) which is used with the BacT/ALERT microbial detection system in qualitative procedures for enhanced recovery and detection of aerobic and facultative anaerobic micro organisms. The blood culture bottles were then transported to the department of Microbiology where culture and sensitivity tests were done. In all patients only the first episode of bacteremia was included for further analysis.

A study form was completed which included the patients demographic details, co-morbidities and the possible source of infection (community acquired or nosocomial). The history of prior antibiotic use and the details of antibiotics used for the

current episode of bacteremia were noted. The antibiotics were prescribed by the treating physician and the choice was not influenced by the study.

Severity of acute illness was assessed at the time of the positive blood culture by using the PITT bacteremia score,[99,103] which is a validated scoring system that is based on mental status, vital signs, requirement for mechanical ventilation, and recent cardiac arrest. Severity of illness was graded within 48 hours of taking the blood culture. Patients accumulating 4 or more points were defined as severely ill. The scoring system is included under annexure 1.

Severity of illness in patients in the intensive care unit at the time of bacteremia was assessed by using the SAPS II scoring system,[100-102],which includes 15 variables including 12 physiological variables. These were assessed with in 48 hours of detection of a positive blood culture for *Klebsiella* or *E coli*. Using these variables, the predicted mortality was for the current illness was calculated for all patients admitted in the various ICU s (MICU, SICU, and TICU) at the time of detection of bacteremia. The SAPS II is shown under Annexure 2. Calculation of the score was done by the help of a SAPS II scoring calculator available on line[102].

The primary source of bacteremia was attributed to a urinary tract, intra-abdominal focus, and pneumonia or, if a focus could not be identified, classified as a primary bloodstream infection. The primary source of bacteremia was localized to the above sites if the same organism could be isolated from a fluid specimen from that site or based on clinical signs and imaging studies.

Follow up:

The patients were followed up for 2 weeks after the diagnosis of bacteremia to assess the clinical outcome.

Microbiological methods:

The blood culture sample taken from the study patients at the onset of fever were inoculated into the BacT/ALERT blood culture bottles, which contain 22 ml of complex culture media and 8 ml of a 6.5 % charcoal suspension. An inoculated bottle is placed into the instrument where it is incubated and continuously monitored for the presence of micro organisms. If micro organisms are present in the test sample, carbon dioxide is produced as the organisms metabolize the substrates in the culture medium and the colour of the gas permeable sensor installed at the bottom of the culture bottle changes from blue green to yellow. If a positive blood culture is detected, it is taken out of the machine and a gram stain done. Gram negative bacilli are inoculated into Blood agar, Mc Conkey agar and Lysine agar tube and direct antibiotic susceptibility testing is done. Antibiotic susceptibility was tested in all the blood culture isolates which grew *Klebsiella* and *E coli* by the disc diffusion test, which is the initial screening test for production of ESBL. All the isolates resistant to either cefotaxime or ceftazidime by the disc diffusion test were tested for phenotypic confirmation of ESBL production, by the double disc diffusion test according to the CLSI performance standards current as of 2006[78,88]. The criteria for classification as ESBL producing isolate was a $\geq$ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone. E coli ATCC25922 and Klebsiella pneumoniae ATCC700603 strains were used as control organisms throughout the study.

The results of culture sensitivity were reported as Susceptible(S), Intermediate(I) and Resistant(R) based on CLSI guidelines.[78]

Statistical methods:

Data entry was done using the Statistical Package for the Social Sciences (SPSS) software package (version 11). Descriptive statistics were calculated using SPSS software. Chi-square test was used for comparison of categorical variables. Odds ratio (OR) and confidence intervals (CI) were calculated and a 'p' value less than 0.05 was considered statistically significant. All reported p values are two-sided. Univariate analysis was performed to assess the risk factors for clinical outcome among he study patients.

The study design and methods were approved by the Fluid Research Committee, Christian Medical College, Vellore

Definitions:

- **Nosocomial bacteremia**: defined as *Klebsiella spp* and *E.coli* bacteremia occurring among patients more than 48 hours after admission to CMCH or another hospital or among those patients who had an invasive procedure done (minor surgical procedure, intravenous administration of drugs or placement of a urinary catheter) and the bacteremia was attributable to that procedure.
- **An episode of bacteremia** is defined as the period of 14 days from the time of collection of the first blood culture positive for the above bacteria.
- **Previous antibiotic therapy** is defined as antibiotics given for at least 2 days within the 14 days before an episode of *Klebsiella spp* or *E. coli* bacteremia.
- **Mortality** was death from any cause within 14 days from the date of the first positive blood culture for *Klebsiella* spp and *E. coli*.
- **An appropriate antibiotic** was defined as an antibiotic to which the bacterial isolate was susceptible in vitro.

Table 5 : Baseline characteristics

Characteristic	Data
Demographic data	
Median age	49(years)
Male	78(59.54)
Female	53(40.46)
Primary department to which patient was admitted n(%)	
General Medicine	44(33.59)
Hematology	21(16.0)
Other medical specialties	20(12.27)
Gastroenterology	16(12.2)
General surgery	17(12.96)
Other surgical specialties	13(9.92)
Source of bacteremia	
Urinary tract	59(45.04)
Intra abdominal	29(22.14)
Pneumonia	7(5.34)
Undetermined	36(27.48)
Co morbidities	
Type II diabetes mellitus	56(42.7)
Hypertension	28(21.4)
Chronic renal failure	11(8.4)
Chronic liver disease	13(9.9)
Solid tumors	10(7.6)
Hematological malignancies	20(15.3)
HIV infection	4(3.1)
Recent surgery	23(17.6)
Ischemic heart disease	9 (6.9)
Cerebrovascular accident	4(3.1)

RESULTS

Population characteristics:

One hundred and thirty one episodes of bacteremia were included in the study during the period of 4 months from Feb 2007 to May 2007, the baseline characteristics are shown in table 5. Seventy eight (59.54%) of the subjects were males.

Figure 5 : Department wise distribution of patients

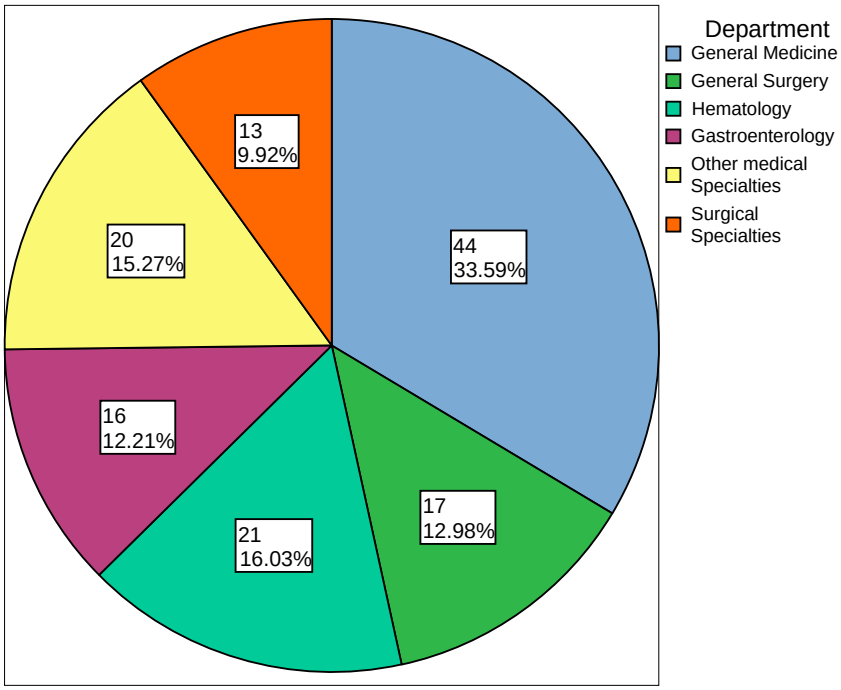
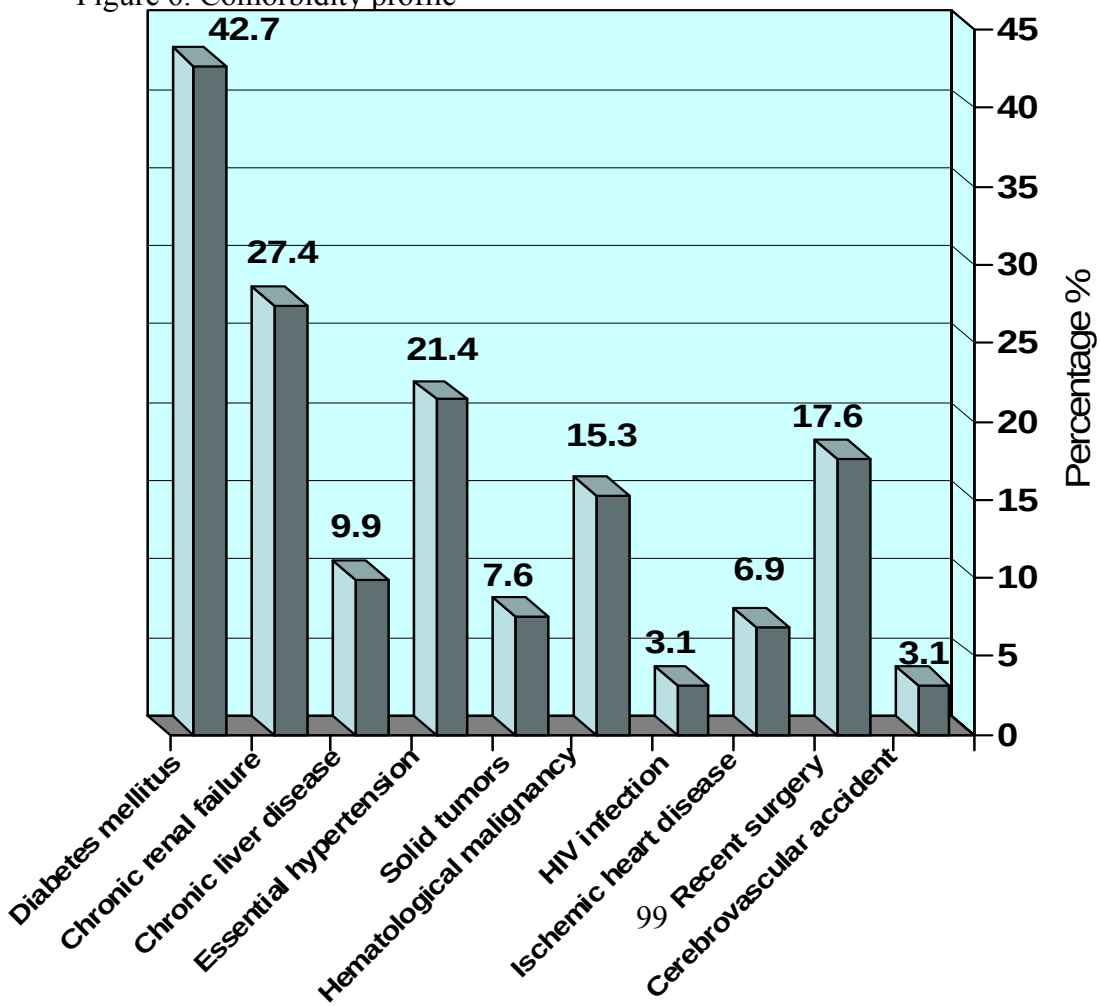


Figure 6. Comorbidity profile



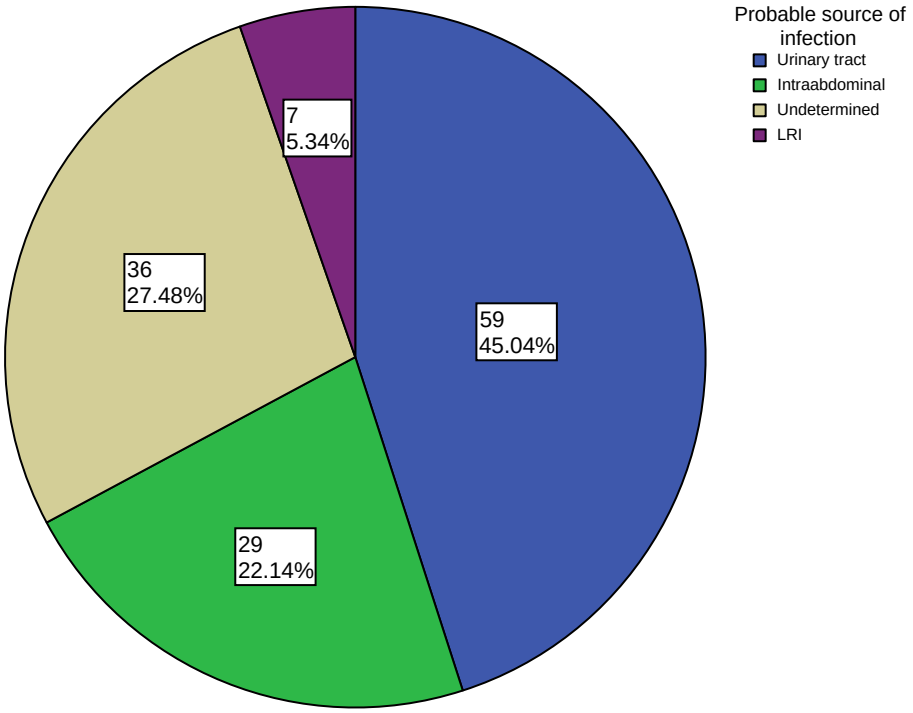
The patients were admitted to the various departments, shown in figure 5. Out of the 131 episodes of bacteremia, there were 10 admissions to the Intensive care units (8 in MICU & 2 in SICU) and the rest, in various wards of the hospital. The primary department from which the patients were admitted to the ICU is shown in table 6.

Table 6. ICU admissions

Department	No. of admissions
General Medicine	4
General surgery	2
Medical specialties	2
Surgical specialties	2

The comorbidity profile is shown in figure 6. The most common co-morbid condition was diabetes mellitus type 2, seen in 56(42.7%) patients. Multiple co-morbidities (2 or more) were seen in 39.7 % (52/131) of patients. 45.8 % (60/131) of the patients had only 1 co-morbidity while 14.5 5% (19/131) did not have any.

Figure 7. Source of infection



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The most common primary source of the bacteremia was the urinary tract, seen in 59(45.04%) patients. An intra-abdominal source was identified in 29(22.14%) patients, and pneumonia was the source of bacteremia in 7(5.34%). In 36(27.48%) patients, the source could not be identified. This is shown in figure 7.

Majority of patients with bacteremia of undetermined origin had a hematological malignancy where primary blood stream infection is well known to occur.

Figure 8: Distribution -Community acquired Vs Nosocomial bacteremia

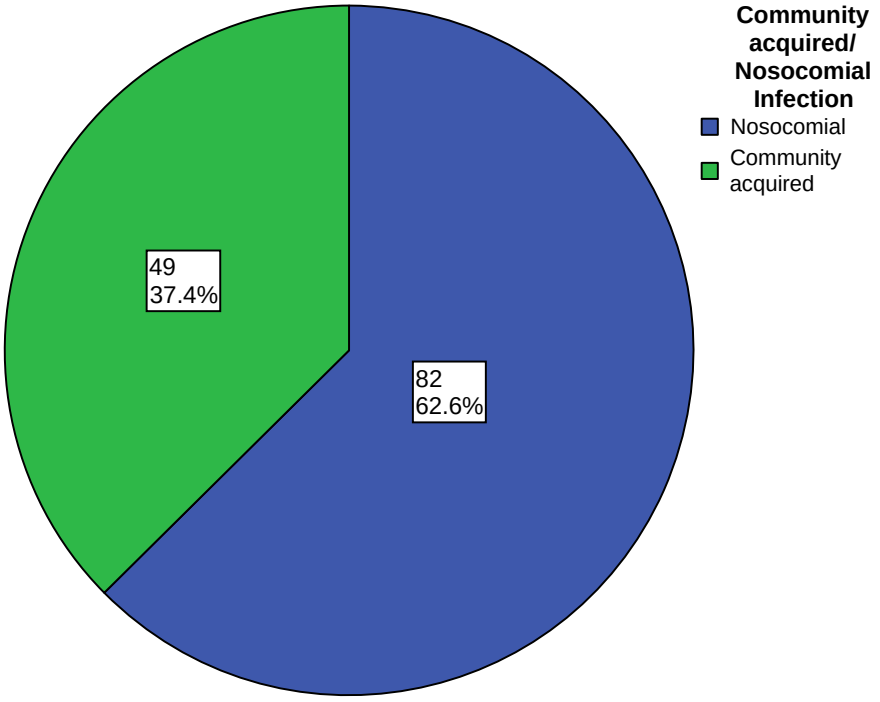
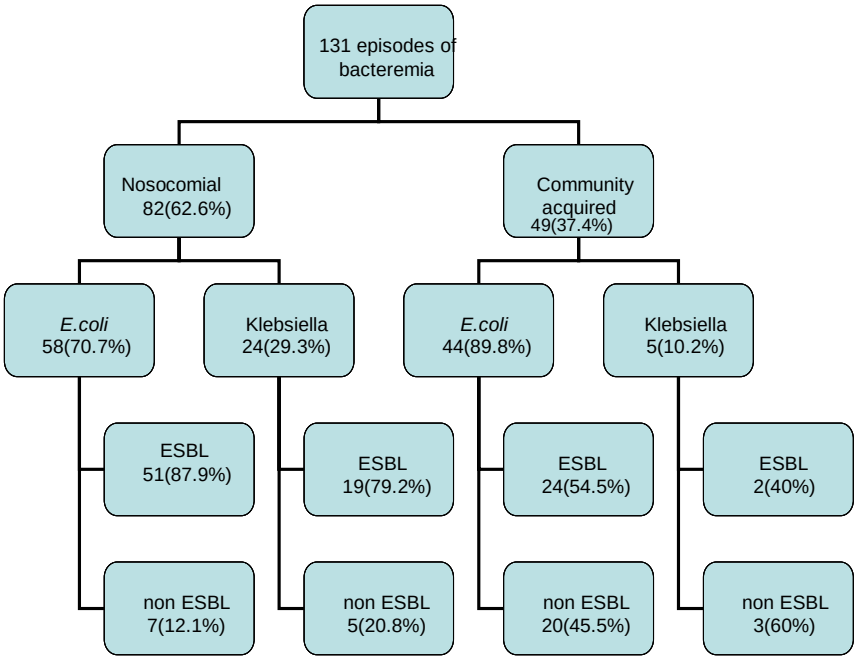


Figure 9: ESBL production in the 131 episodes of bacteremia



Eighty two (62.6%) of the episodes of bacteremia were nosocomially acquired, figure 8.

The comparison of comorbidity profile of patients with nosocomial and community acquired infections is shown in table 7. ESBL production among these isolates is shown in figure 9.

Nosocomial infections were significantly more common in patients who had undergone recent surgery (p=0.002) and in those with solid organ tumors (p = 0.011).

Table 7: Comparison of comorbidity profile and source of infection between Nosocomial and Community acquired bacteremia.

Comorbidity	Nosocomial infection n = 82	Community acquired infection n = 49	p value
Diabetes Mellitus	31(37.8)	25(51.0)	0.139
Chronic renal failure	8(9.8)	3(6.1)	0.468
Chronic liver disease	6(7.3)	7(14.3)	0.197
Hypertension	17(20.7)	11(22.4)	0.817
Solid tumors	10(12.2)	0 (0)	**0.011**
Hematological malignancy	15(18.3)	5(10.2)	0.213
HIV infection	3(3.7)	1(2.0)	0.603
Ischemic heart disease	5(6.1)	4(8.2)	0.651
Cerebrovascular accident	2(2.43)	2(4.1)	0.597
Recent surgery	21(25.6)	2(4.1)	**0.002**
Source of infection			
Urinary tract	31(37.8)	28(57.1)	0.154
Intra- abdominal	19(23.2)	10(20.4)	0.154
Pneumonia	5(6.1)	2(4.1)	0.154
Undetermined	27(32.9)	9 (18.4)	0.154

Figure 10 : Percentage of bacteremias caused by *E coli* and *Klebsiella*

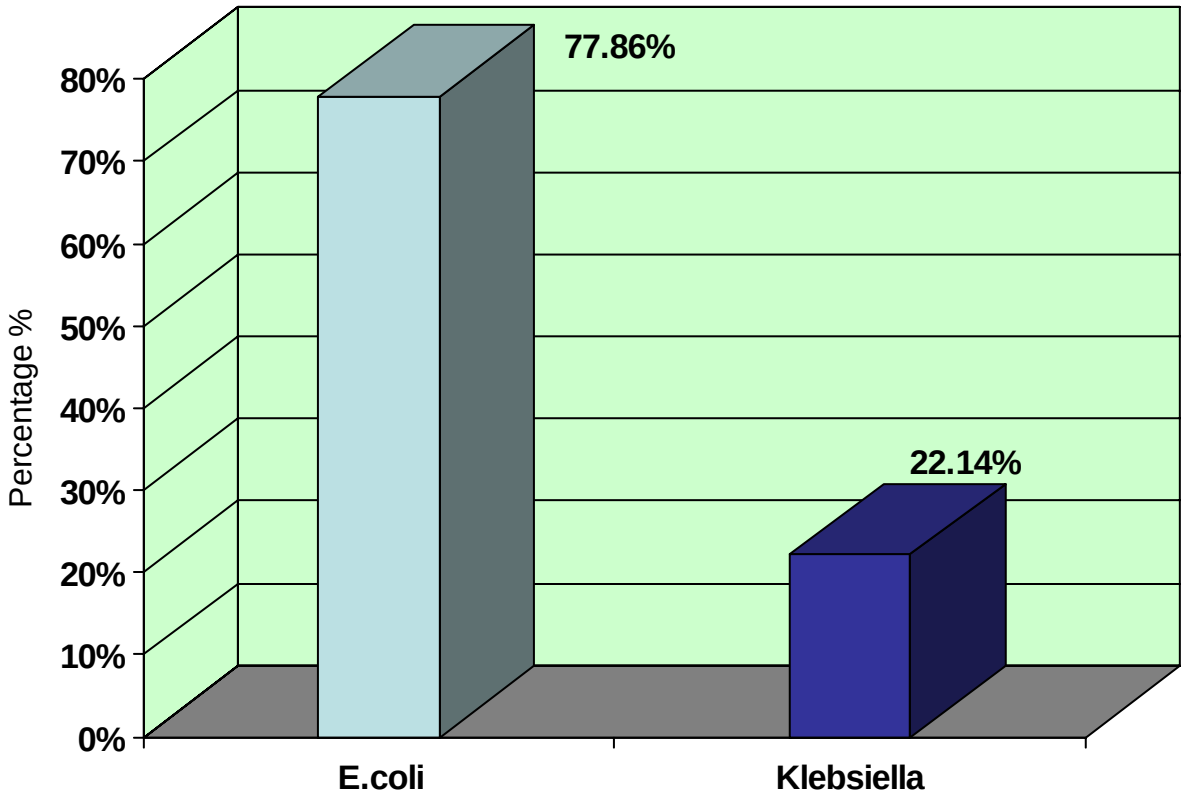


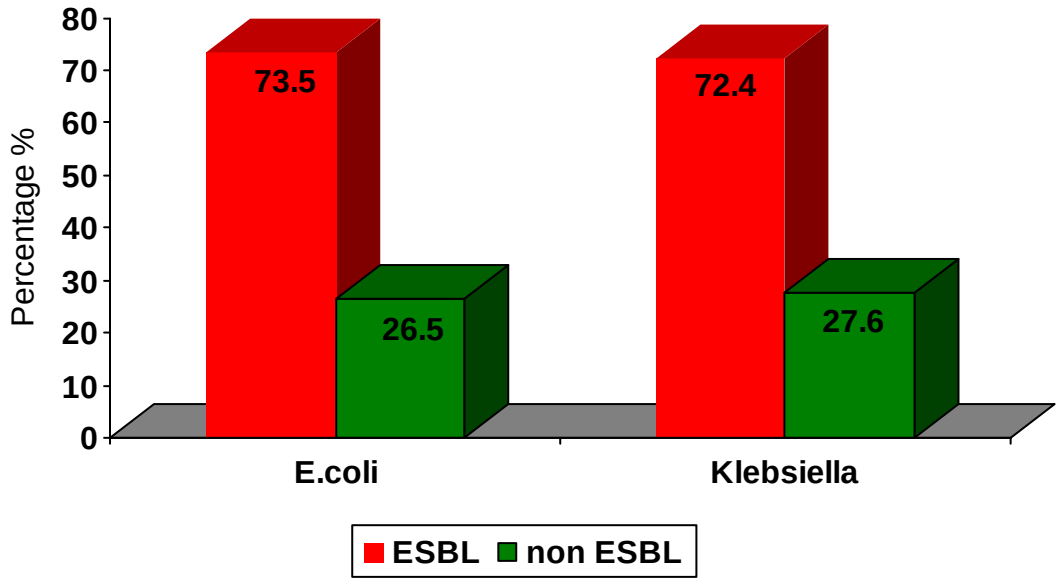
Table 8. Primary source of bacteremia.

Organism	*E.coli* n (%)	*Klebsiella* n (%)
Urinary tract	59(57.8)	0
Intra- abdominal	17(16.7)	12(41.4)
Pneumonia	3(2.9)	4(13.8)
Undetermined	23(22.6)	13(44.8)
Total	102(100)	29(100)

Microbiological data:

E coli caused 102(77.86%) of the episodes of bacteremia and Klebsiella 29(22.14%), shown in figure 10, the source of bacteremia for these two groups is shown in table 8.

Figure 11. ESBL producing isolates.



In the initial screening test by disc diffusion, 73.5% of *E coli* isolates and 72.4% of *Klebsiella* isolates were resistant to Cefotaxime and Ceftazidime, shown in figure 11 and all these isolates were confirmed to produce ESBL by the double disc diffusion test.

The sensitivity of the *E coli* and *Klebsiella* isolates to Carbapenems was 99.2 %, to the 3rd and 4th generation Cephalosporins 26.7%, to the beta lactams and beta lactamase inhibitors, ranged between 18.3 to 32.8%.

The resistance profile of all the isolates to the common antibiotics is shown in figures 12, 13 and 14.

Figure 12..Antibiotic resistance profile for all isolates

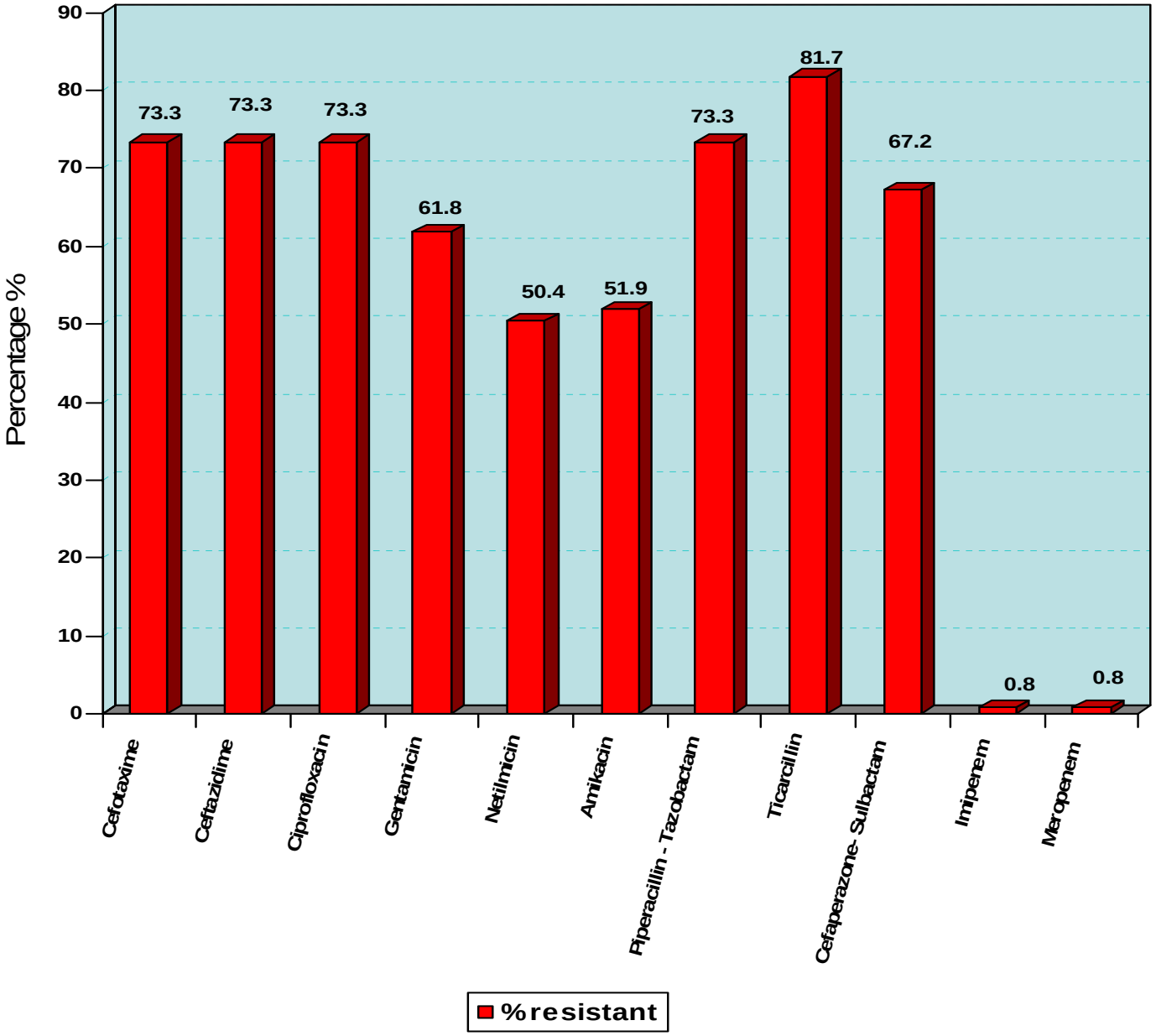


Figure 13.Antibiotic resistance profile for ESBL producing isolates:

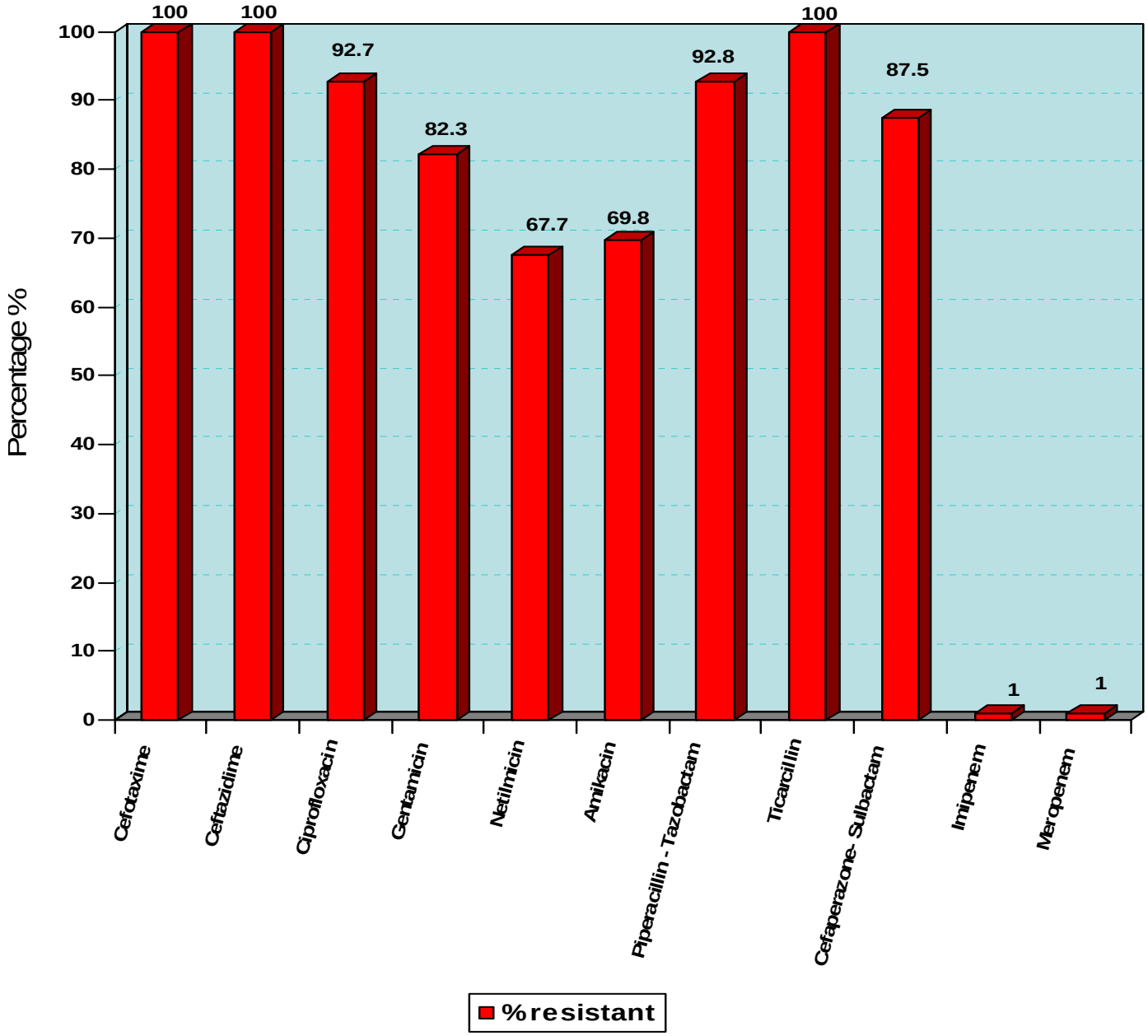


Figure 14. .Antibiotic resistance profile for non ESBL producing isolates

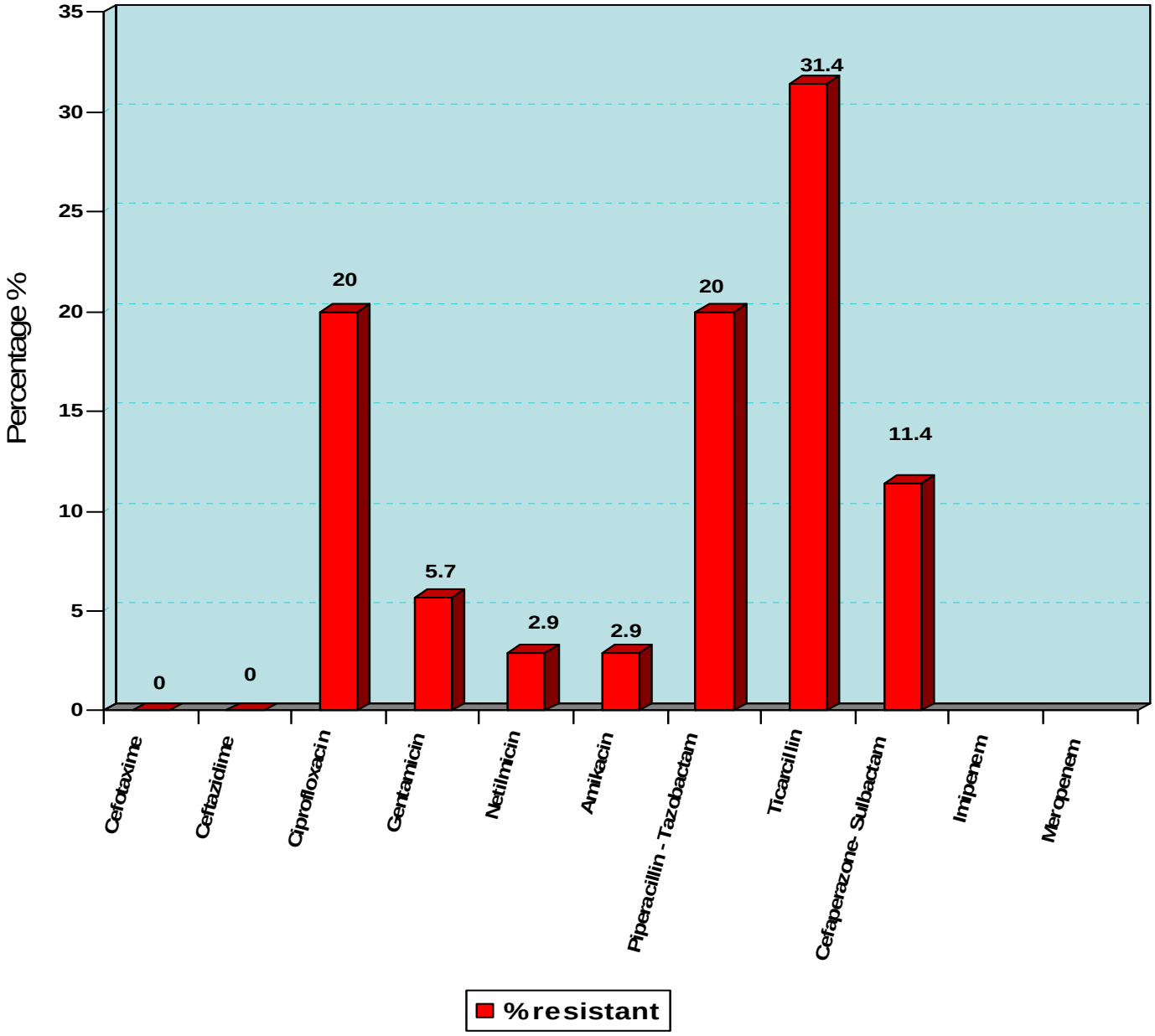


Figure 15. ESBL producing isolates

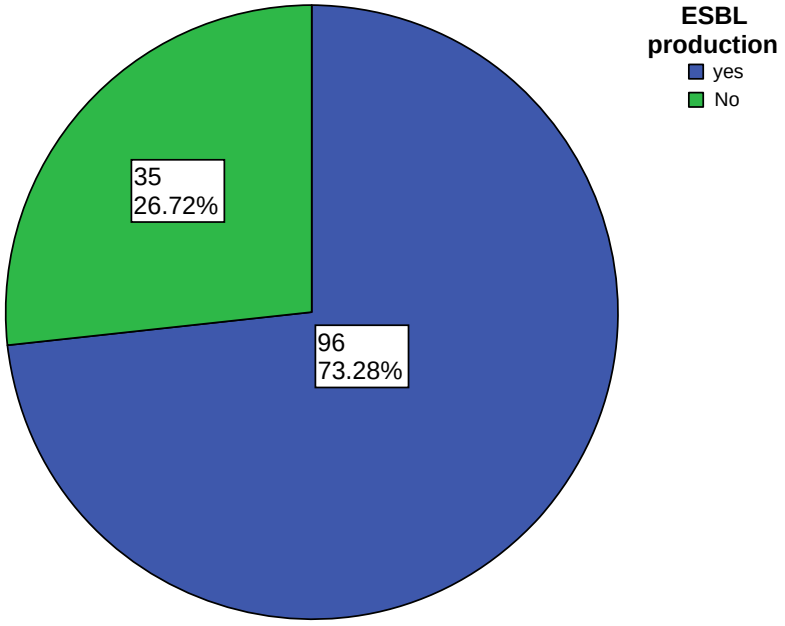


Table 9. ESBL Vs non ESBL, characteristics

Patient characteristics	ESBL producing isolates 96(100 %)	Non ESBL producing isolates 35(100 %)	p
Median age (years)	50	45	0.508
Sex (M:F)	61:35	17:18	0.122
Primary source			
Urinary tract	44(45.8%)	15(42.9%)	0.319
Intra abdominal	19(19.8%)	10(28.6%)	
Pneumonia	7(7.3%)	0	
Undetermined	26(27.1%)	10(28.6%)	
Nosocomial infections n(82)	70	12	**<0.001**
Comorbidity			
Diabetes mellitus	44(45.8)	12(34.3)	0.237
Chronic renal failure	8(8.3)	3(8.6)	0.965
Chronic liver disease	7(7.3)	6(17.1)	0.095
Hypertension	21(21.9)	7(20)	0.817
Solid tumors	7(7.3)	3(8.6)	0.807
Hematological malignancy	14(14.6)	6(17.1)	0.719
HIV infection	3(3.1)	1(2.9)	0.937
Ischemic heart disease	5(5.2)	4(11.4)	0.213
Cerebrovascular accident	2(2.1)	2(5.7)	0.285
Recent surgery	17(17.7)	6(17.1)	0.940

ESBL production was seen in 96(73.28%) patients (figure 15). ESBL production was observed in 75 out of 102(73.5%) patients with *E.coli* bacteremia as compared to 21 out of 29(72.4%) with *Klebsiella* bacteremia, figure11. This difference was not statistically significant (p=0.905).

Bacteremia due to ESBL producing *E coli* and *Klebsiella* spp were significantly more common in nosocomially acquired infections when compared to community acquired infections(85.37% Vs 53.06% p= <0.001). There was no significant difference in the median age, sex ratio or co morbidity profile among patients with and with out ESBL producing bacteremias, table 9.

Figure 16. Use of antibiotics over the previous two weeks.

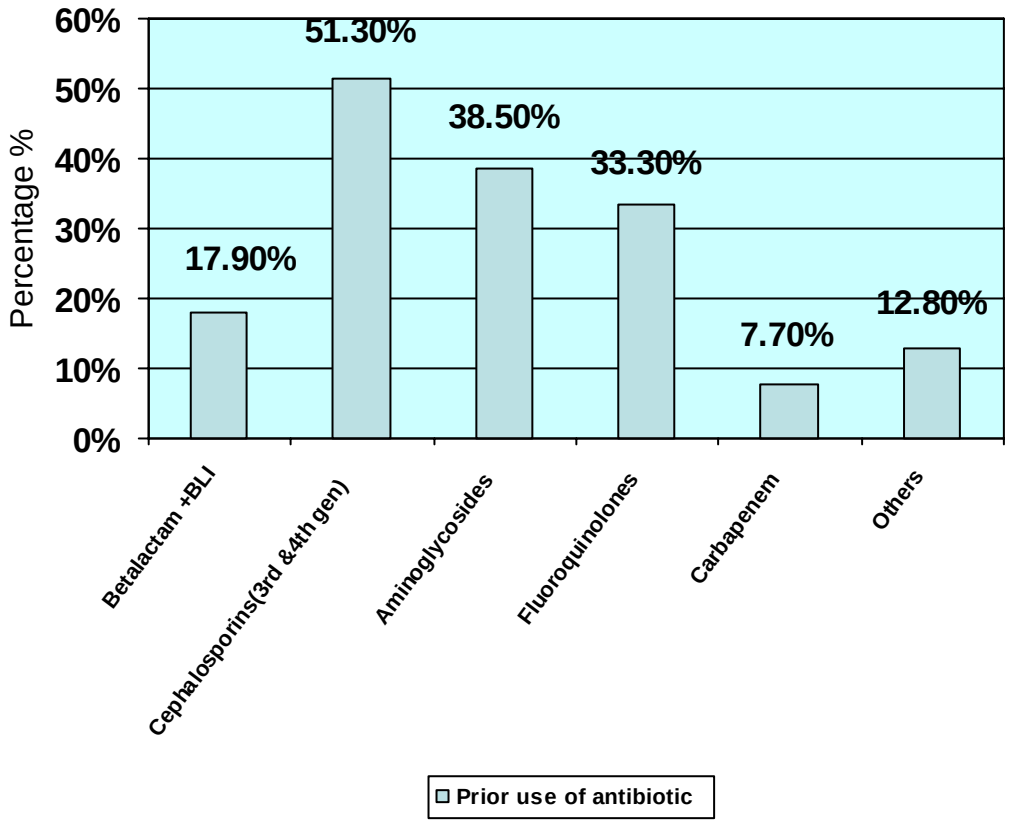
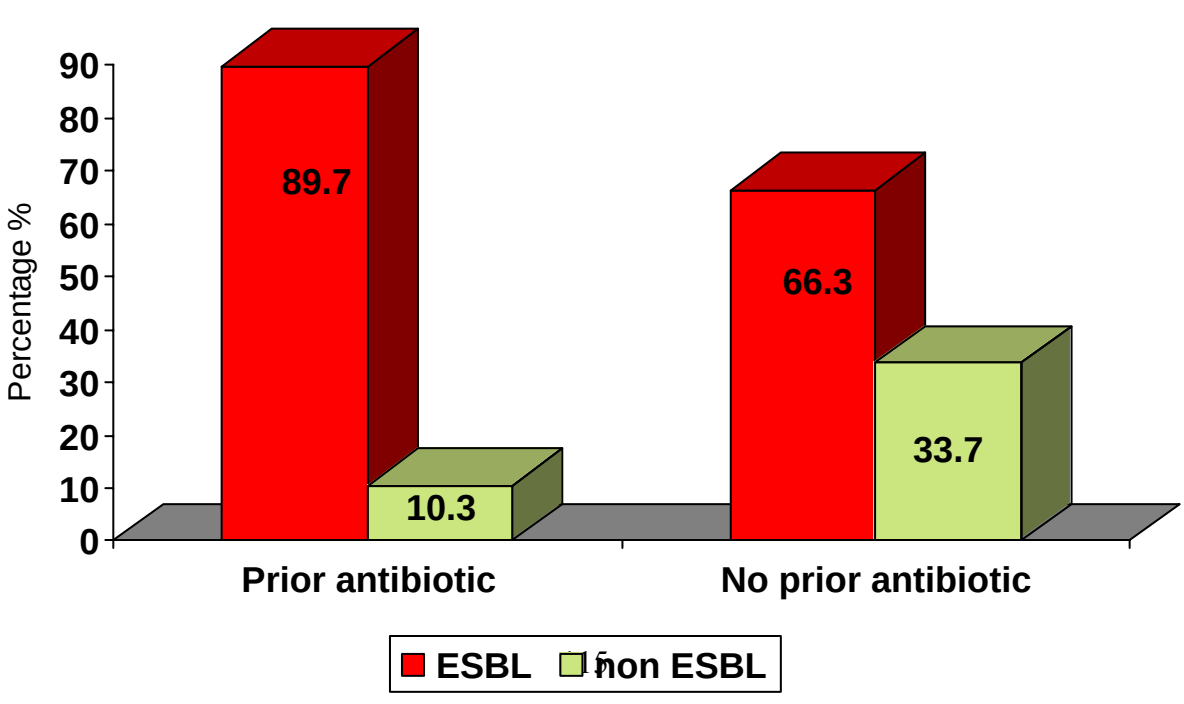


Figure 17: ESBL production in relation to use of prior antibiotics



Prior antibiotic use:

Thirty nine (29.8%) patients gave history of antibiotic use in the preceding two weeks. The most common antibiotics used were 3rd and 4th generation cephalosporins 20(51.3%). The others were beta lactams with or without betalactam inhibitors 7(17.9%), aminoglycosides15 (38.5%), fluoroquinolones, 13(33.3%), carbapenems 3(7.7%) and others, 5(12.8%).

Bacteremia caused by ESBL producing isolates was significantly higher (p = 0.006) among patients who had received any antibiotic in the preceding 2 weeks, table 10. The breakup according to the individual classes of antibiotics is shown in table 11. The prior use of 3rd or 4th generation cephalosporins was associated (p=0.017) with ESBL production among isolates of *Klebsiella* and *E coli* causing bacteremia.

Table 10 Prior antibiotic use and ESBL production

p = 0.006		ESBL production		Total
		Yes	No	
Prior Antibiotic	Used	35	4	39
	Not used	61	31	92
Total		96	35	131

Table 11. Prior antibiotic use and ESBL production- breakdown of antibiotics used

Prior administration of	ESBL (n = 96)	Non ESBL(n=35)	p
Beta lactam ± Beta lactam inhibitors (7)	6	1	0.445
Cephalosporins (3rd and 4th generation) (20)	19	1	0.017
Fluoroquinolones (13)	12	1	0.102
Aminoglycosides (15)	14	1	0.062
Carbapenems (3)	3	0	0.292

Figure 18. Use of prior antibiotic:

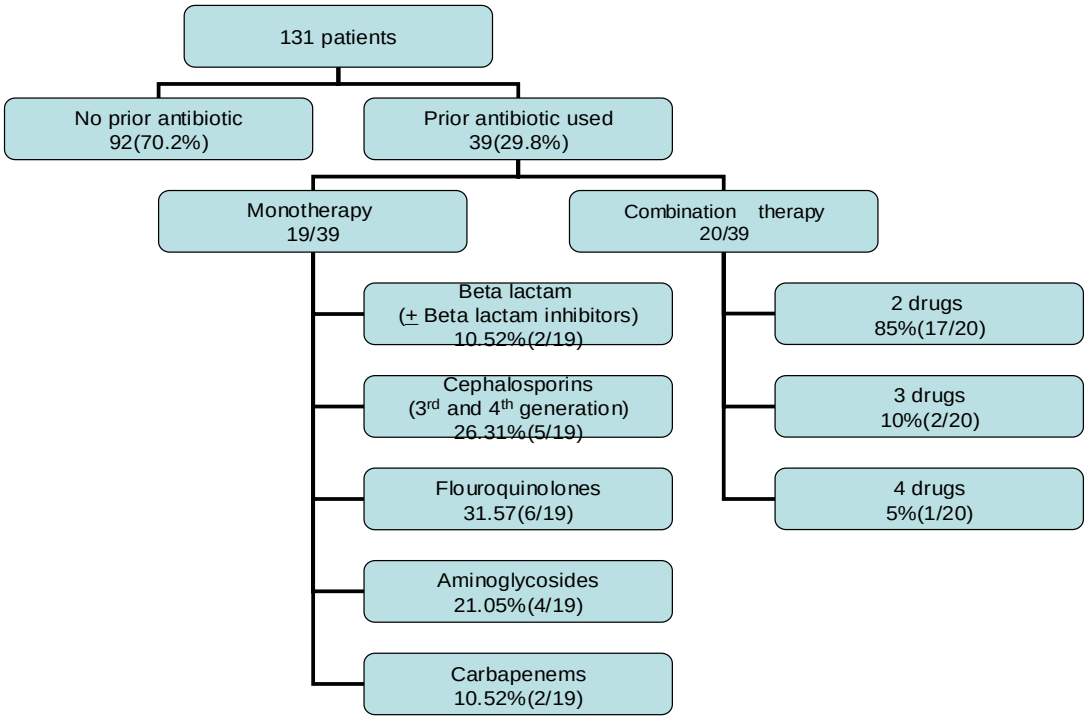
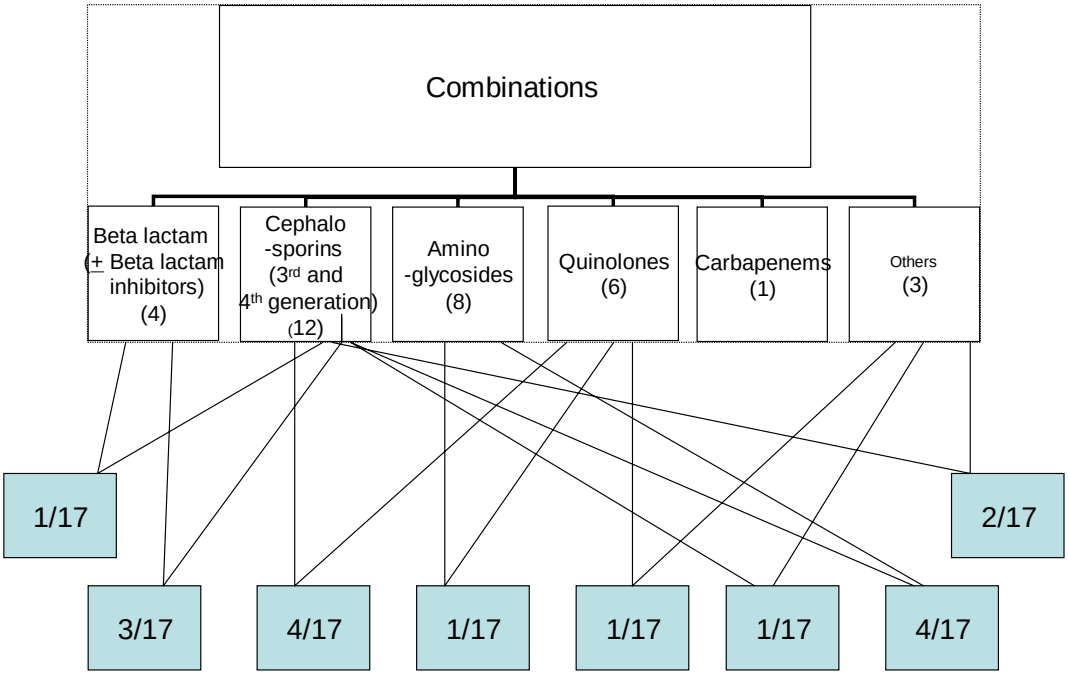


Figure 19. Combinations used



The antibiotic combinations are shown in figures 18 and 19.

Figure 20. Choice of initial antibiotic

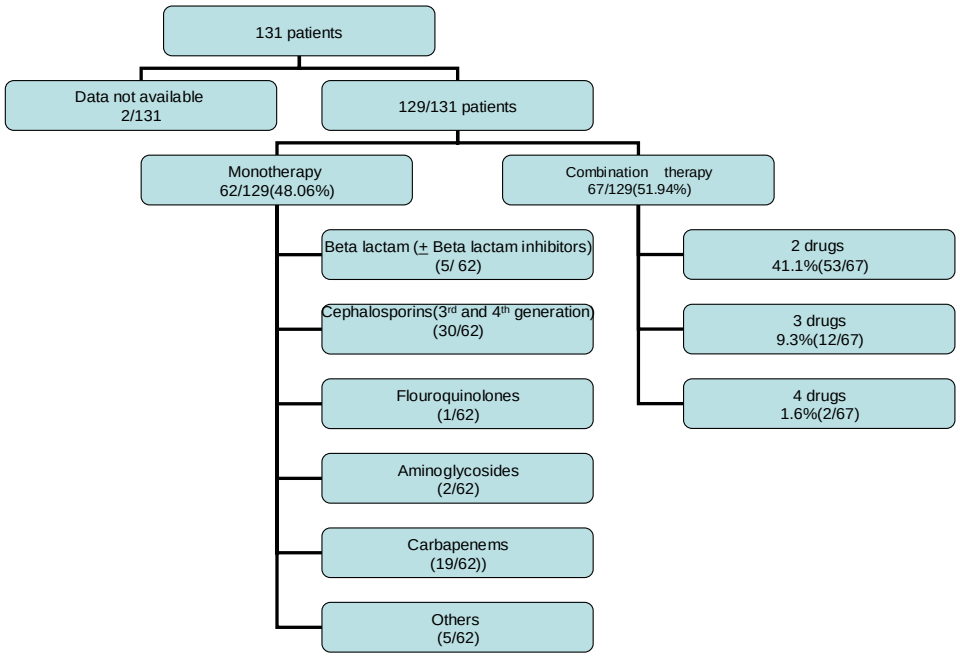
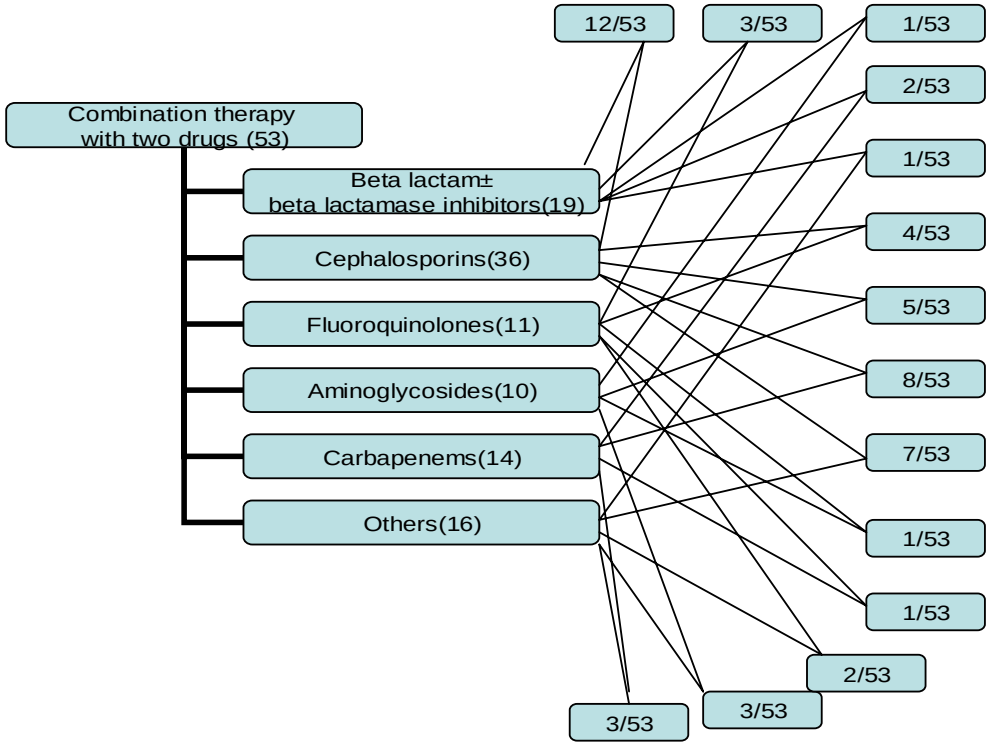


Figure 21.Combination therapy with two antibiotics



Antibiotic choices and combinations used for the initial treatment of the bacteremias is shown in figures 20,21 and 22. Monotherapy was chosen in 62 episodes (48.06%), the commonest being 3rd and 4th generation cephalosporins in 23.25 % and combination therapy in 67 episodes (51.94%).

Figure 22 . Groups of antibiotics used as the initial therapy

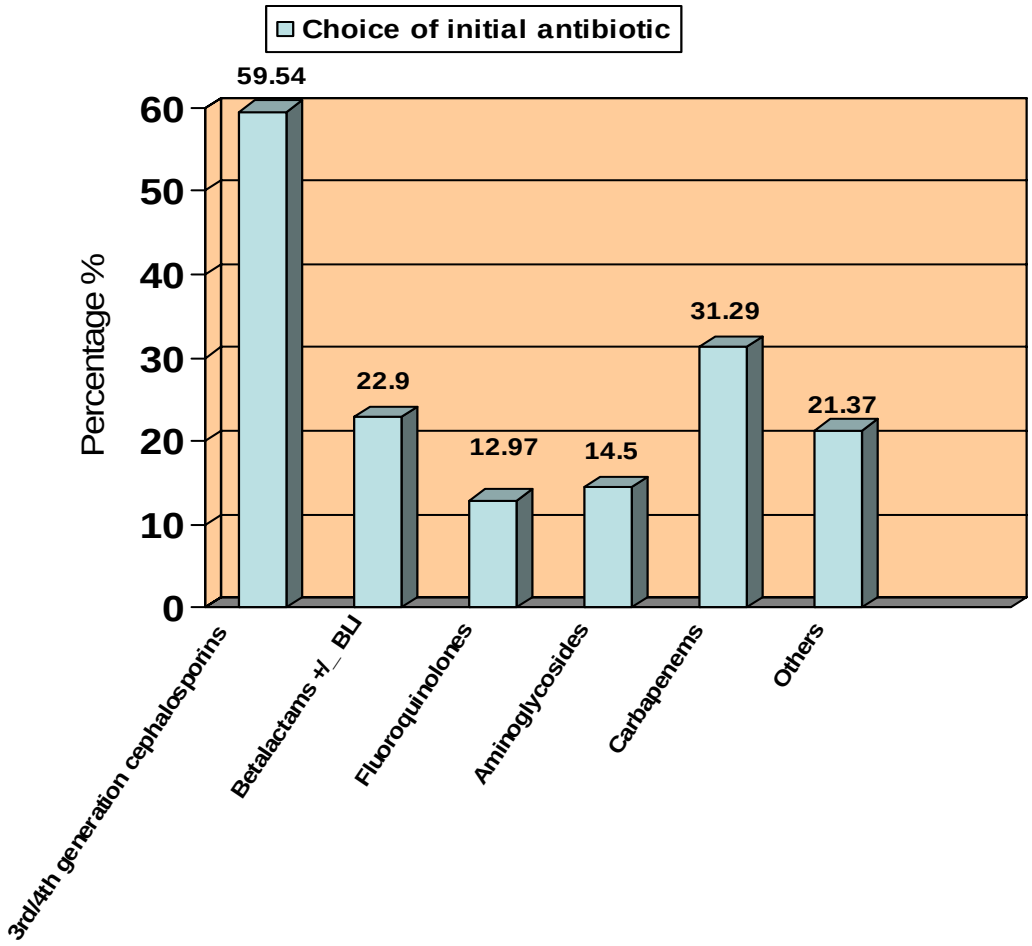


Figure 23. Appropriateness of Initial choice of antibiotics

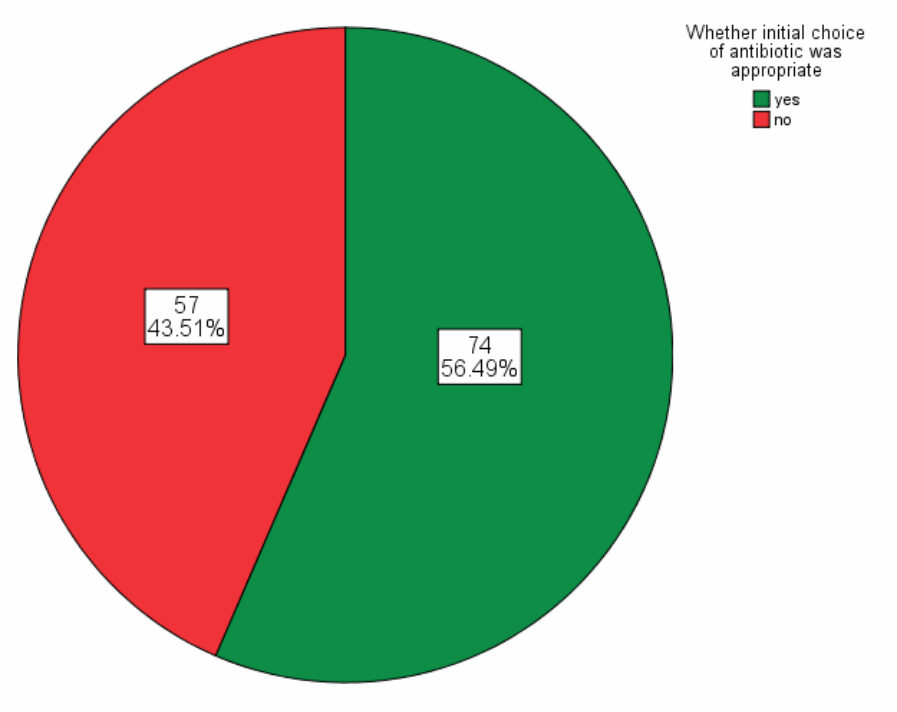
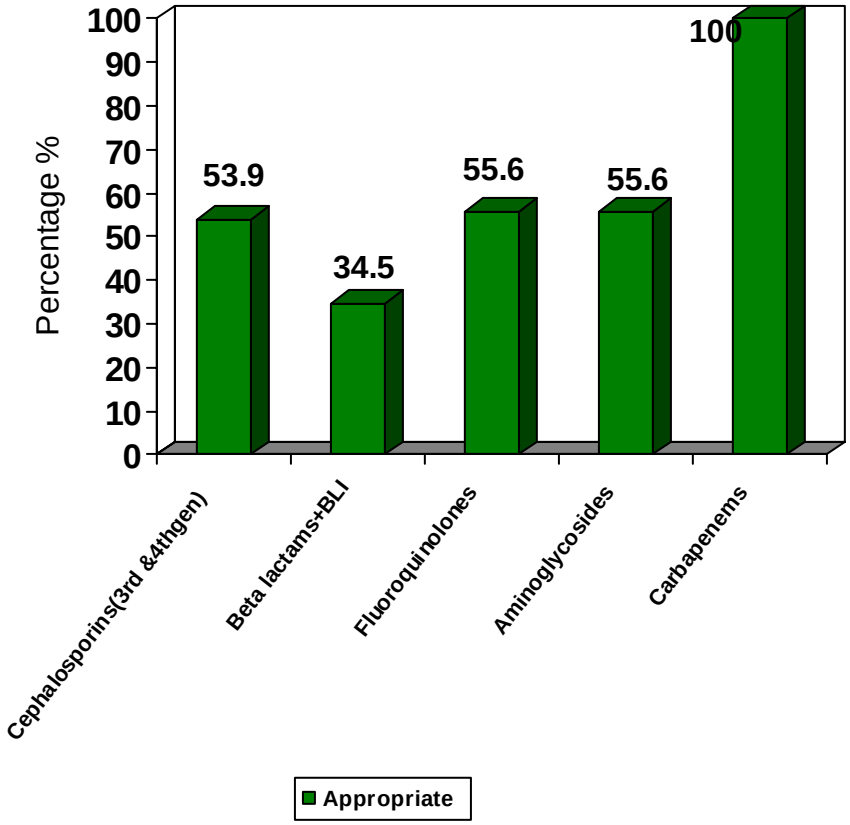


Figure 24 .Appropriateness according to class of antibiotics



Appropriateness of Initial choice of antibiotic:

The antibiotic choice for the bacteremic episode was termed appropriate based on the in vitro susceptibility report in 74(56.49%) episodes, figure 23.

The most appropriate choice were carbapenems(41/41). The corresponding rates for 3rd and 4th generation cephalosporins was 53.9 %(41/76), aminoglycosides and fluoroquinolones was 55.6 %(10 / 18) and for betalactams with or without betalactam inhibitors was 34.5 %(10/29), this data is shown in figure 24.

Figure 25. Out come after 2 weeks

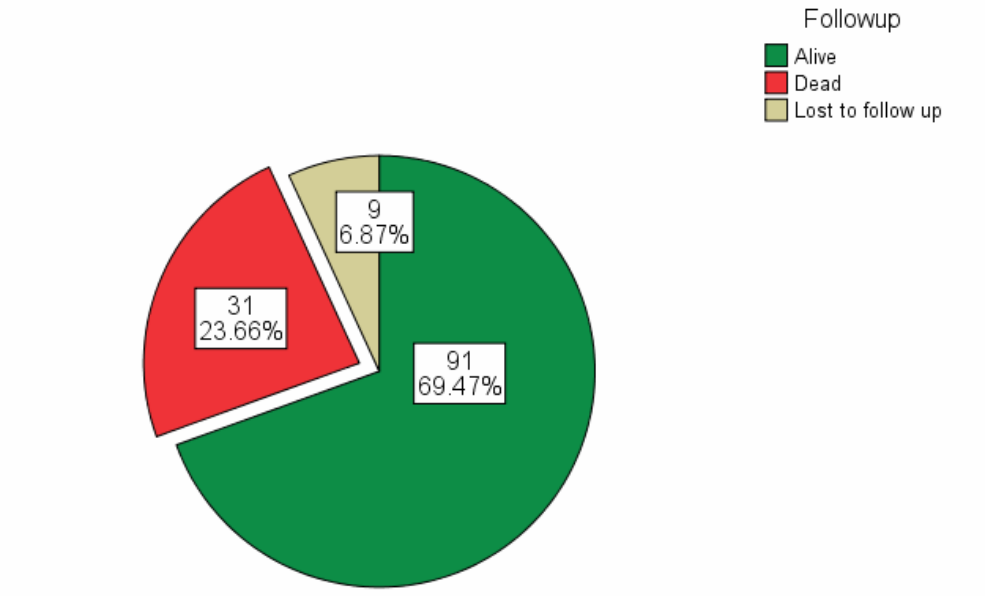


Figure 26 :Outcome among patients with ESBL producing isolates

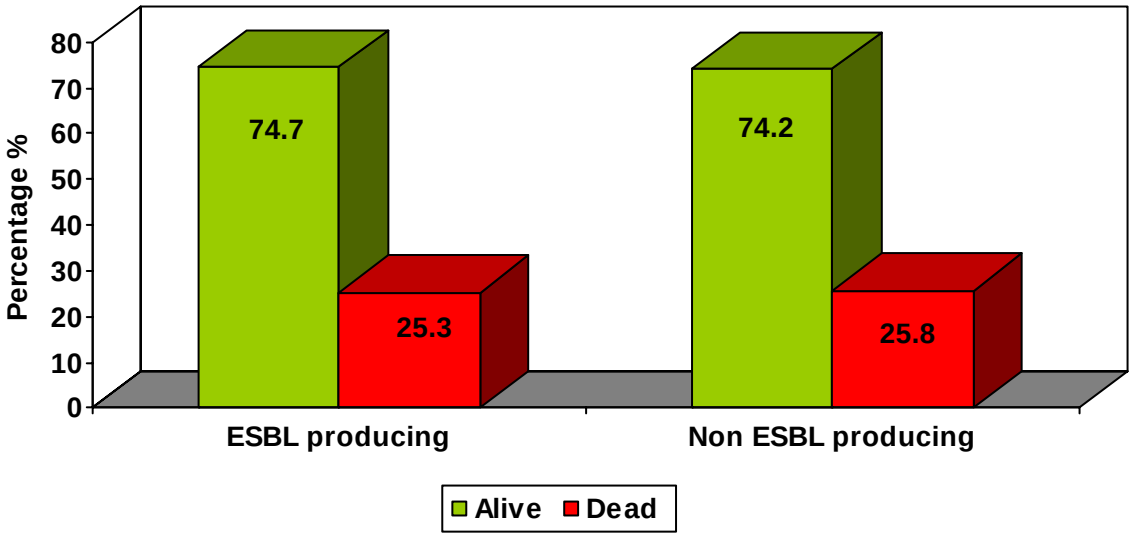


Table 12. Mortality based on source of infection

p = 0.005	Follow up		Total
	Alive	Dead	
Urinary tract	46	9	55
Intra abdominal	16	10	26
Undetermined	27	7	34
Pneumonia	2	5	7
Total	91	31	122

Outcome measures:

One hundred and twenty two patients were followed up for 14 days from the time of diagnosis of a bacteremia. Of the 122, 74.6 %(91) were alive and 25.4 %(31) were dead at 14 days, (figure 25). In those patients admitted to the ICUs, the mortality rate was 60% (6 out of 10). This was higher than in those not admitted to the ICUs, (p = 0.009).

ESBL production was the same among patients admitted to the ICU, 7(70%) and in those admitted to the general wards, 89(73.5%) p = 0.807. There was no significant difference between ESBL and non ESBL producing infections with regard to mortality (p 0.953),(figure 26)

There was no significant difference in the mortality between nosocomial Vs community acquired infections (p= 0.443), *E coli* Vs *Klebsiella* (p=0.842), appropriateness of the initial choice of antibiotic (p= 0.520) or the use of carbapenems as initial therapy (p=0.968). Higher mortality was seen in patients with a pneumonia (p=0.005),(table 12).

Figure 27. Duration of hospital stay

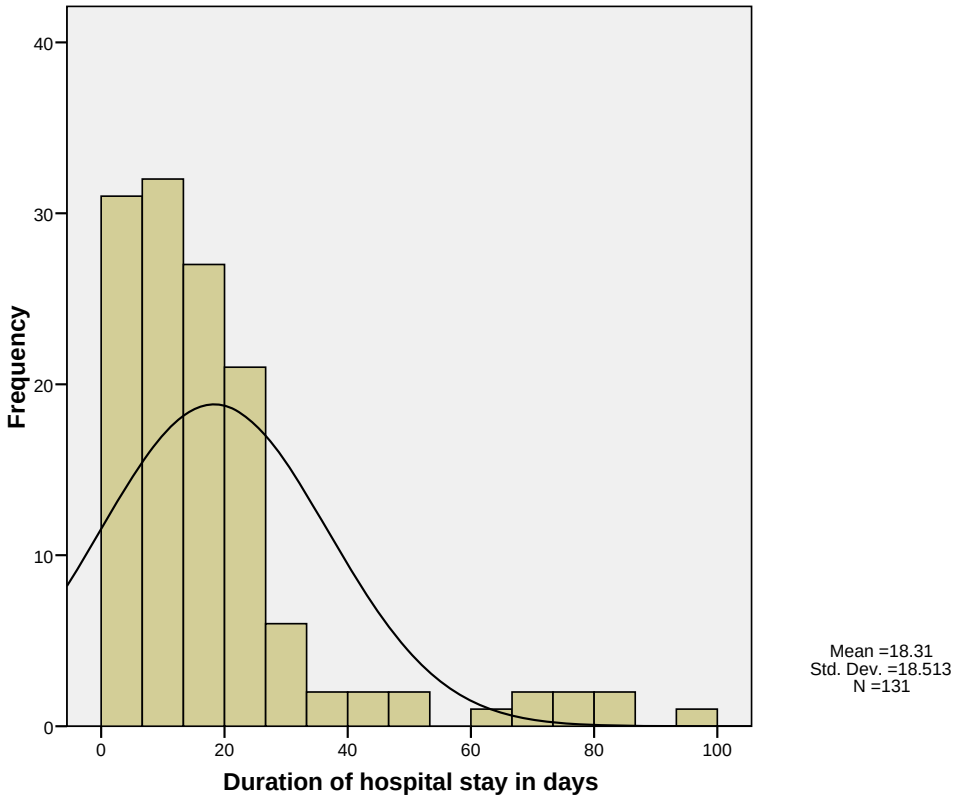


Table 13. Duration of hospital stay

Total duration of hospitalization		ESBL producing	Non ESBL producing
Mean		20.29	12.86
Median		15.50	9.00
Mode		2	9
Std. Deviation		19.587	14.028
Range		99	77
Minimum		1	1
Maximum		100	78
Percentiles			
	25	8.25	5.00
	50	15.50	9.00
	75	22.00	14.00

The median duration of hospital stay was 14.00 and the mean was 18.31± 18.513 days,(figure 27). The minimum duration was 1 day, with a maximum of 100 days.

The average duration of hospitalization was higher in patients with infections caused by ESBL producing isolates (20.29±19.59 days) as compared to patients with infections due to non ESBL strains (12.86±14.03) p=0.042. The median duration for the two groups was 15.50 and 9 days respectively. This is shown in table 13.

The overall duration of stay among patients with nosocomial infections (23.63±21.09), was predictably longer than those with community acquired infections (9.39±6.95). This was significant at a p value of < 0.001.

The mean duration of ICU stay was longer in patients with infections caused by ESBL producing isolates , 21.57 ± 22.38 days , as compared to 8.33 ± 5.03 days, in patients with infections due to sensitive strains. However, this difference did not achieve statistical significance (p = 0.355), probably due to the very small number of patients studies, 7 and 3 in the two groups respectively.

Table 14 . PITTS bacteremia score

Mean		1.71
Median		1.00
Mode		0
Std. Deviation		2.585
Variance		6.684
Range		11
Minimum		0
Maximum		11
Percentiles	25	.00
	50	1.00
	75	2.00

Severity scoring:

The median PITT score was 1.77, with a mean of 1.71 ± 2.585, and a maximum value of 11. The PITTS score in patients admitted to the ICUs was significantly higher, 4.70 ± 2.31, as compared to the patients admitted in the other wards, 1.46 ± 2.46, the difference was significant at a p value of < 0.001. Also the PITTS score was significantly lower in patients alive at 14 days of follow up, 0.79 ± 1.15, as compared to 4.81± 3.45, p <0.001.

The PITTS score was higher in community acquired infections, 2.33 ± 2.96, as compared to nosocomial infections, 1.34 ± 2.27, p = 0.049..

The PITTS score in patients with ESBL was not significantly higher, 1.83 ± 2.602, as compared to the patients without ESBL, 1.37 ± 2.545, p = 0.368.

DISCUSSION

Extended spectrum β-lactamase production is the defense mechanism developed by bacteria in the race for survival. This has largely been aided by faulty and inconsistent prescription practices, the world over. The gravity of the situation is more obvious in resource poor settings where antimicrobial prescription policies are either not in place or not respected.

The need remains to quantify this problem, to identify the risk factors for ESBL production, the current antibiotic practices for appropriateness and to assess the risk factors for poor clinical outcome among patients with ESBL producing *E. coli* or *Klebsiella* infection. This has been the main objective of our study.

One hundred and thirty one episodes of bacteremia due to *E. coli* or *Klebsiella* spp were included in the study during the period of 4 months from February 2007 to May 2007. The reason that we chose bacteremic patients as the study population is to avoid the difficulty in differentiating infection and colonization in other samples (e.g. sputum, urine, endotracheal aspirate etc).

The commonest primary source of the bacteremia was the urinary tract followed by an intra abdominal source. The urinary tract was the most common source of infection in other studies also.[7] In a significant number of patients the source of bacteremia could not be determined. Most of them had a hematological malignancy where primary blood stream infection is well known to occur.

ESBL production

With the rapid spread of ESBL producing strains in hospitals all over the world, it is necessary to know the prevalence in a hospital so as to formulate a policy of appropriate antibiotic therapy and other measures to control the spread of the pathogens.. ESBL production was seen in 73.3% of patients with *E. coli* and *Klebsiella* bacteremia which is higher than the reported prevalence in other hospitals in India and other countries. A study done at AIIMS in 2002 showed that 68 % of the E*nterobacteriaceae* isolates produce ESBL and the prevalence in various hospitals abroad range from 3.3% to 86.6% . In intensive care units in which antibiotic use is heaviest and the potential for person to person transmission of organisms is greatest, the prevalence of ESBL producing isolates was the same as that in other wards (73.5 % Vs 70 %) in contrast to other studies, where ICU admissions were associated with an increased risk of infection caused by ESBL producing strains. [7,4]

The unusually high prevalence of ESBL producers in the present series may partly be explained by the fact that the hospital serves as a tertiary referral center in South India and by the fact that most of the ESBL producing isolates were nosocomial in origin. ESBL production was seen in 70 out of 82(85.37%) isolates in nosocomial infection, as compared to community acquired infections, in which only 26 out of 49(53.06%) produced ESBL. The high prevalence of ESBL production among community acquired isolates is alarming when compared to the prevalence rates in other studies. ESBL production among community acquired isolates was only 3.5% in a study done by Paterson et al.[7]

This could be attributable to the following reasons. Excessive use of 3rd and 4th generation cephalosporins in the community could increase the prevalence of resistant strains. Secondly, due to poor quality of drinking water supplied to the community, spread of *Enterobacteriaceae* is difficult to control. In a study done by Mathai,[42] healthy volunteers in Jawadhi hills, a rural district of Vellore, who were antibiotic naïve were found to have a 27.1% fecal carriage rates of ESBL producing E. coli.

Risk factors for ESBL production

Our data has identified previous treatment with 3rd or 4th generation cephalosporins as the most important risk factor associated with ESBL production which is in accordance with the study done by Du B et al[8] and a study done by Lautenbach et al.[75] Other studies have also shown an increase in ESBL production with prior administration of β-lactam antibiotics containing an oxyimino group.[7] However, the relatively few patients who received these preclude definitive conclusions about this link. Our analyses therefore can only provide hypotheses for further investigation with a larger sample size.

Previous antibiotic exposure may lead to resistance to *E coli* and *Klebsiella* because these antibiotics may exert a selective pressure as to eliminate all the sensitive strains rather than through induction of Amp C β-lactamases. 3rd and 4th generation cephalosporins represent the most commonly used antibiotic class in hospitals therefore exerting a predominant selective pressure for development of resistance. Our results confirm the fact that ESBL s may emerge as a result of excessive cephalosporin

use and also indicate that interventions designed to restrict cephalosporin use in order to reduce the level of antibiotic resistance merits further investigation. Some investigators have demonstrated the positive effect of restriction of cephalosporin use on reduction of antibiotic resistance, although not without adverse effects.[104]. It has also been speculated that replacing cephalosporins with antibiotics containing ß-lactamase inhibitors (such as piperacillin–tazobactam) may help to reduce the occurrence of ESBL-producing organisms[93,105].

Because many recent resistance problems can be traced to excessive use of broad-spectrum antibiotics, antibiotic control policies must be considered mandatory to minimize antibiotic resistance. Practical approaches to antibiotic control include restricting the use of particular agents, specifically, defining indications for use or cycling classes of antibiotics to limit the selective pressure on nosocomial flora.

Antibiotic resistance

A high degree of resistance to multiple classes of antibiotics was noted especially to the 3rd and 4th generation cephalosporins and β-lactam and β-lactamase inhibitor combinations (73.3% to cephalosporins and ranging between 67.2-81.7 % to β-lactam and β-lactamase inhibitor combinations).These results were comparable to those of a study done at The National Institute of Communicable Diseases in New Delhi in 2004,[45] which showed a high frequency of resistance among *K. pneumoniae* for the cephalosporins (cefoxitin, cefuroxime, cefotaxime, ceftazidime, and cefepime) and non-cephalosporins (aztreonam, piperacillin, chloramphenicol and trimethoprim-

sulfamethoxazole),in the range of 39.2-88.0% and 51.0-90.2% respectively. Aminoglycosides were found to be the next best choice to carbapenems, as resistance rates were lower (51.9 % - 61.8 %). Significantly, carbapenem resistance was seen in 1 patient, who had a nosocomial infection with a ESBL producing strain of *E coli*, the source of infection being the urinary tract. He had an indwelling urinary catheter and acquired the infection after 50 days of hospitalization.

Initial antibiotic choice:

With the widespread use of extended-spectrum cephalosporins throughout the world, strains that produce ESBLs have been detected on every inhabited continent. The emergence and spread of ESBL-producing strains have led to questions regarding the optimal therapy for infections caused by ESBL-producing strains.

We evaluated the appropriateness of the initial antibiotics used for each episode of bacteremia and found that the antibiotic chosen for the bacteremic episode was appropriate in 56.49%.This figure is much less than that seen in a retrospective study done at the Department of Critical Care Medicine, Peking Union Medical College Hospital,[8] where antibiotic treatment was considered appropriate in 83% of the cases.

In addition to poor choice of antibiotics in approximately half the episodes of bacteremia, many patients were given multiple antibiotics as initial therapy (3 antibiotics in 9.3 % and 4 drugs in 1.6%) which is irrational. In our study, only 48.6 % of the patients received monotherapy with the choice being appropriate in only 56.49 %

where as studies done in other hospitals have shown 83 % accuracy with 89 % of the episodes of bacteremia given monotherapy.[8] The need to revise our policy on antibiotic administration is evident.

Clinical outcome

The mortality rate after 14 days of onset of *Klebsiella* and *E. coli* bacteremia was 31/122 (23.66 %) with 9 (6.87 %) patients lost to follow up. A similar mortality rate was seen in a study done by Paterson et al[7] It has been reported that multi drug resistant bacteria may result in a much higher mortality among patients with the infection.[7],[36] However we have found a similar mortality rate between patients with ESBL and non ESBL producing strains causing bacteremia (25.3% Vs 25.8 %; p= 0.953), both the groups having a similar co-morbidity profile. Some studies done before also did not show any association between ESBL production and a poor clinical outcome.[8]

This may be attributed to the fact that approximately half the patients did not receive appropriate antibiotic. The initial antibiotic choice was appropriate in only 43.75% of the patients with bacteremia due to ESBL producing isolates where as 91.42 % of the patients with non ESBL producing isolates received an appropriate antibiotic; $p<0.001$. Therefore, our study suggests that ESBL producing bacteria may be associated with an increased risk of inappropriate antibiotic treatment due to the multi drug resistant characteristics of the pathogen, which in turn, may lead to treatment failure and patient death.

In the study by Du B et al,[8] antibiotic treatment failure was the only independent risk factor for hospital mortality (OR 15.376, P=0.001).However, contrary to expectations and previous data, our study did not show a worse outcome among patients who received inappropriate antibiotic for the episode of bacteremia.. This, again, can be attributed to be multi drug resistance among ESBL producing isolates which predominate the study isolates, the small sample size and the lack of knowledge of the genotype of the isolates..

It is postulated that the advantage of carbapenems over other antibiotics due to their stability against the hydrolytic effects by different β-lactamases including ESBL and Amp C may contribute to a better outcome when used as the initial antibiotic of choice for the episode of bacteremia . However, we found that the use of a carbapenem as the initial antibiotic did not affect the clinical outcome, though in other studies,this had a positive effect on outcome.[5,7,8]

Clearly, a lot of improvement is still needed in the following fields:

1. High index of suspicion of gram negative bacteria producing ESBL, especially among nosocomially acquired infections.
2. Judicious use of antibiotics, especially 3rd and 4th generation cephalosporins to decrease the chance of bacteria developing resistance
3. Administration of appropriate empiric antibiotic for each episode of a gram negative infection.

LIMITATIONS OF THE STUDY

1. Being a tertiary care centre with referral status, the data presented here is not likely to be representative of the general population .So while hospital based infection control practices may be improved upon based on the recommendations presented here, studies looking at regional antimicrobial susceptibility patterns will be a more definite step to control the menace of antimicrobial resistance.
2. Documentations of prior antibiotic administration was suboptimal and this reflected on the quality of subset analysis.
3. Nine patients (6.87%) were lost to follow up
4. The subset of patients admitted to ICU was small; hence the applicability of the SAPS II score as a prognostic tool could not be assessed. Further, the mortality and morbidity correlates of this group could not be characterized.

CONCLUSIONS

One hundred and thirty one sequentially encountered adult patients with *E coli* or *Klebsiella* bacteremia were studied and followed up prospectively over a period of 2 weeks to assess clinical outcome.

1. ESBL production was observed in 73.28% of the isolates with 53.06 % of the community acquired infection and 85.36% of nosocomial infections caused by ESBL producing strains.
2. Prior use of antibiotics, especially 3rd and 4th generation cephalosporins were associated with an increased risk of infection by ESBL producing isolates of *E coli* and *Klebsiella*
3. A very high rate (50.4% -81.7%) of resistance to other major classes of antibiotics (e.g. fluoroquinolones, aminoglycosides, β-lactams and β-lactamase inhibitors) was observed, with carbapenems being the only class of drug with a good activity against ESBL producing Enterobacteriaceae. That, resistance to carbapenems was also noted among the isolates, is a cause of concern for the future.
4. Initial antibiotics were inappropriate in 53% (based on antibiotic susceptibility profile).
5. The mortality following the episode of bacteremia was not influenced by the initial choice of the antibiotic. However, carbapenems should be the choice of initial empiric therapy for serious life threatening infections caused by ESBL producing

Enterobacteriaceae (with de-escalation when culture and sensitivity reports are available).

Reference List

(1) Nathisuwan S, Burgess DS, Lewis JS. Extended-spectrum beta-lactamases: epidemiology, detection, and treatment. *Pharmacotherapy.* 2001;21:920-928.

(2) Medeiros AA. Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. *Clin Infect Dis.* 1997;24 Suppl 1:S19-45.:S19-S45.

(3) Jacoby GA, Munoz-Price LS. The new beta-lactamases. *N Engl J Med.* 2005;352:380-391.

(4) Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum beta lactamase producing gram negative bacteria in a tertiary care hospital. *Indian J Med Res.* 2002;115:153-7.:153-157.

(5) Schiappa DA, Hayden MK, Matushek MG et al. Ceftazidime-resistant Klebsiella pneumoniae and Escherichia coli bloodstream infection: a case-control and molecular epidemiologic investigation. *J Infect Dis.* 1996;174:529-536.

(6) Pena C, Pujol M, Ricart A et al. Risk factors for faecal carriage of Klebsiella pneumoniae producing extended spectrum beta-lactamase (ESBL-KP) in the intensive care unit. *J Hosp Infect.* 1997;35:9-16.

(7) Paterson DL, Hujer KM, Hujer AM et al. Extended-spectrum beta-lactamases in Klebsiella pneumoniae bloodstream isolates from seven countries: dominance and widespread prevalence of SHV- and CTX-M-type beta-lactamases. *Antimicrob Agents Chemother.* 2003;47:3554-3560.

(8) Du B, Long Y, Liu H et al. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae bloodstream infection: risk factors and clinical outcome. *Intensive Care Med.* 2002;28:1718-1723.

(9) Chaudhary U, Aggarwal R. Extended spectrum -lactamases (ESBL) - An emerging threat to clinical therapeutics. *Indian J Med Microbiol.* 2004;22:75-80.

(10) Spratt BG, Cromie KD. Penicillin-binding proteins of gram-negative bacteria. *Rev Infect Dis.* 1988;10:699-711.

(11) Novak R, Charpentier E, Braun JS, Tuomanen E. Signal transduction by a death signal peptide: uncovering the mechanism of bacterial killing by penicillin. *Mol Cell.* 2000;5:49-57.

(12) Gold HS, Moellering RC, Jr. Antimicrobial-drug resistance. *N Engl J Med.* 1996;335:1445-1453.

(13) Pitout JD, Sanders CC, Sanders WE, Jr. Antimicrobial resistance with focus on beta-lactam resistance in gram-negative bacilli. *Am J Med.* 1997;103:51-59.

(14) Tomasz A. Antibiotic resistance in Streptococcus pneumoniae. *Clin Infect Dis.* 1997;24 Suppl 1:S85-8.:S85-S88.

(15) Mulligan ME, Murray-Leisure KA, Ribner BS et al. Methicillin-resistant Staphylococcus aureus: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. *Am J Med.* 1993;94:313-328.

(16) Nordmann P. Trends in beta-lactam resistance among Enterobacteriaceae. *Clin Infect Dis.* 1998;27 Suppl 1:S100-6.:S100-S106.

(17) Thomson KS, Smith ME. Version 2000: the new beta-lactamases of Gram-negative bacteria at the dawn of the new millennium. *Microbes Infect.* 2000;2:1225-1235.

(18) Joshi SG, Litake GM, Ghole VS, Niphadkar KB. Plasmid-borne extended-spectrum beta-lactamase in a clinical isolate of Acinetobacter baumannii. *J Med Microbiol.* 2003;52:1125-1127.

(19) Vahaboglu H, Ozturk R, Aygun G et al. Widespread detection of PER-1-type extended-spectrum beta-lactamases among nosocomial Acinetobacter and Pseudomonas aeruginosa isolates in Turkey: a nationwide multicenter study. *Antimicrob Agents Chemother.* 1997;41:2265-2269.

(20) Weldhagen GF, Poirel L, Nordmann P. Ambler class A extended-spectrum beta-lactamases in Pseudomonas aeruginosa: novel developments and clinical impact. *Antimicrob Agents Chemother.* 2003;47:2385-2392.

(21) Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother.* 1995;39:1211-1233.

(22) Sawai T, Mitsuhashi S, Yamagishi S. Comparison of the chromosomal and extrachromosomal genetic determinants controlling staphylococcal penicillinase production. *Jpn J Microbiol.* 1968;12:531-533.

(23) Richmond MH, Sykes RB. The beta-lactamases of gram-negative bacteria and their possible physiological role. *Adv Microb Physiol.* 1973;9:31-88.:31-88.

(24) Hirai K, Sato K, Matsubara N, Katsumata R, Inoue M, Mitsuhashi S. Immunological properties of beta-lactamases that hydrolyze cefuroxime and cefotaxime. *Antimicrob Agents Chemother.* 1981;20:262-264.

(25) Ambler RP. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci.* 1980;289:321-331.

(26) Huang W, Petrosino J, Hirsch M, Shenkin PS, Palzkill T. Amino acid sequence determinants of beta-lactamase structure and activity. *J Mol Biol.* 1996;258:688-703.

(27) Nukaga M, Mayama K, Hujer AM, Bonomo RA, Knox JR. Ultrahigh resolution structure of a class A beta-lactamase: on the mechanism and specificity of the extended-spectrum SHV-2 enzyme. *J Mol Biol.* 2003;328:289-301.

(28) Knox JR. Extended-spectrum and inhibitor-resistant TEM-type beta-lactamases: mutations, specificity, and three-dimensional structure. *Antimicrob Agents Chemother.* 1995;39:2593-2601.

(29) Sirot D, Recule C, Chaibi EB et al. A complex mutant of TEM-1 beta-lactamase with mutations encountered in both IRT-4 and extended-spectrum TEM-15, produced by an Escherichia coli clinical isolate. *Antimicrob Agents Chemother.* 1997;41:1322-1325.

(30) Kuzin AP, Nukaga M, Nukaga Y, Hujer AM, Bonomo RA, Knox JR. Structure of the SHV-1 beta-lactamase. *Biochemistry.* 1999;38:5720-5727.

(31) Yuan M, Aucken H, Hall LM, Pitt TL, Livermore DM. Epidemiological typing of klebsiellae with extended-spectrum beta-lactamases from European intensive care units. *J Antimicrob Chemother.* 1998;41:527-539.

(32) Yong D, Shin JH, Kim S et al. High prevalence of PER-1 extended-spectrum beta-lactamase-producing Acinetobacter spp. in Korea. *Antimicrob Agents Chemother.* 2003;47:1749-1751.

(33) Bonnet R, Sampaio JL, Chanal C et al. A novel class A extended-spectrum beta-lactamase (BES-1) in Serratia marcescens isolated in Brazil. *Antimicrob Agents Chemother.* 2000;44:3061-3068.

(34) Jacoby GA, Medeiros AA. More extended-spectrum beta-lactamases. *Antimicrob Agents Chemother.* 1991;35:1697-1704.

(35) Danel F, Hall LM, Duke B, Gur D, Livermore DM. OXA-17, a further extended-spectrum variant of OXA-10 beta-lactamase, isolated from Pseudomonas aeruginosa. *Antimicrob Agents Chemother.* 1999;43:1362-1366.

(36) Chow JW, Fine MJ, Shlaes DM et al. Enterobacter bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med.* 1991;115:585-590.

(37) Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. *Clin Microbiol Infect.* 2002;8:321-331.

(38) Kasper, Braunwald Fauci Hauser. Harrisons principles of internal medicine. Mc Graw Hill company library , 878-886. 2005.
Ref Type: Electronic Citation

(39) Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to Escherichia coli: focus on an increasingly important endemic problem. *Microbes Infect.* 2003;5:449-456.

(40) Gransden WR, Eykyn SJ, Phillips I, Rowe B. Bacteremia due to Escherichia coli: a study of 861 episodes. *Rev Infect Dis.* 1990;12:1008-1018.

(41) Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev.* 1998;11:589-603.

(42) Mathai D. *Clinico-Epidemiologic and Molecular Characterization of MRSA and ESBL Producing Escherichia coli, Klebsiella spp. and Enterobacter spp., Causing Hospital and Community - Acquired Infections in Indian Hospitals* [2007.

(43) Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended-spectrum beta-lactamase-producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. *J Med Microbiol.* 2003;52:421-425.

(44) Jain A, Mondal R. Prevalence & antimicrobial resistance pattern of extended spectrum beta-lactamase producing Klebsiella spp isolated from cases of neonatal septicaemia. *Indian J Med Res.* 2007;125:89-94.

(45) Grover SS, Sharma M, Pasha ST, Singh G, Lal S. Antimicrobial susceptibility pattern and prevalence of extended spectrum beta-lactamase (ESBLs) producing strains of Klebsiella pneumoniae from a major hospital in New Delhi. *J Commun Dis.* 2004;36:17-26.

(46) Burwen DR, Banerjee SN, Gaynes RP. Ceftazidime resistance among selected nosocomial gram-negative bacilli in the United States. National Nosocomial Infections Surveillance System. *J Infect Dis.* 1994;170:1622-1625.

(47) Sirot J, Chanal C, Petit A, Sirot D, Labia R, Gerbaud G. Klebsiella pneumoniae and other Enterobacteriaceae producing novel plasmid-mediated beta-lactamases markedly active against third-generation cephalosporins: epidemiologic studies. *Rev Infect Dis.* 1988;10:850-859.

(48) Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of strains expressing an extended-spectrum beta-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. *Clin Infect Dis.* 2001;32 Suppl 2:S94-103.:S94-103.

(49) Karlowsky JA, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Trends in antimicrobial susceptibilities among Enterobacteriaceae isolated from hospitalized patients in the United States from 1998 to 2001. *Antimicrob Agents Chemother.* 2003;47:1672-1680.

(50) Arlet G, Rouveau M, Casin I, Bouvet PJ, Lagrange PH, Philippon A. Molecular epidemiology of Klebsiella pneumoniae strains that produce SHV-4 beta-lactamase and which were isolated in 14 French hospitals. *J Clin Microbiol.* 1994;32:2553-2558.

(51) Quale JM, Landman D, Bradford PA et al. Molecular epidemiology of a citywide outbreak of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae infection. *Clin Infect Dis.* 2002;35:834-841.

(52) Wiener J, Quinn JP, Bradford PA et al. Multiple antibiotic-resistant Klebsiella and Escherichia coli in nursing homes. *JAMA.* 1999;281:517-523.

(53) Arpin C, Dubois V, Coulange L et al. Extended-spectrum beta-lactamase-producing Enterobacteriaceae in community and private health care centers. *Antimicrob Agents Chemother.* 2003;47:3506-3514.

(54) Radice M, Power P, Di CJ, Gutkind G. Early dissemination of CTX-M-derived enzymes in South America. *Antimicrob Agents Chemother.* 2002;46:602-604.

(55) Woodford N, Ward ME, Kaufmann ME et al. Community and hospital spread of Escherichia coli producing CTX-M extended-spectrum beta-lactamases in the UK. *J Antimicrob Chemother.* 2004;54:735-743.

(56) Moland ES, Black JA, Hossain A, Hanson ND, Thomson KS, Pottumarthy S. Discovery of CTX-M-like extended-spectrum beta-lactamases in Escherichia coli isolates from five US States. *Antimicrob Agents Chemother.* 2003;47:2382-2383.

(57) Shibata N, Doi Y, Yamane K et al. PCR typing of genetic determinants for metallo-beta-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. *J Clin Microbiol.* 2003;41:5407-5413.

(58) Kurokawa H, Yagi T, Shibata N, Shibayama K, Arakawa Y. Worldwide proliferation of carbapenem-resistant gram-negative bacteria. *Lancet.* 1999;354:955.

(59) Igari J, Oguri T, Hiramatsu N, Akiyama K, Koyama T. [Post-marketing surveillance of antibacterial activities of cefozopran against various clinical isolates--I. Gram-positive bacteria]. *Jpn J Antibiot.* 2002;55:1-21.

(60) Sader HS, Biedenbach DJ, Jones RN. Global patterns of susceptibility for 21 commonly utilized antimicrobial agents tested against 48,440

Enterobacteriaceae in the SENTRY Antimicrobial Surveillance Program (1997-2001). *Diagn Microbiol Infect Dis.* 2003;47:361-364.

(61) Tsakris A, Pournaras S, Woodford N et al. Outbreak of infections caused by Pseudomonas aeruginosa producing VIM-1 carbapenemase in Greece. *J Clin Microbiol.* 2000;38:1290-1292.

(62) Cornaglia G, Mazzariol A, Lauretti L, Rossolini GM, Fontana R. Hospital outbreak of carbapenem-resistant Pseudomonas aeruginosa producing VIM-1, a novel transferable metallo-beta-lactamase. *Clin Infect Dis.* 2000;31:1119-1125.

(63) la-Costa LM, Coelho JM, Souza HA et al. Outbreak of carbapenem-resistant Acinetobacter baumannii producing the OXA-23 enzyme in Curitiba, Brazil. *J Clin Microbiol.* 2003;41:3403-3406.

(64) Bradford PA, Bratu S, Urban C et al. Emergence of carbapenem-resistant Klebsiella species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. *Clin Infect Dis.* 2004;39:55-60.

(65) Bisson G, Fishman NO, Patel JB, Edelstein PH, Lautenbach E. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella species: risk factors for colonization and impact of antimicrobial formulary interventions on colonization prevalence. *Infect Control Hosp Epidemiol.* 2002;23:254-260.

(66) Mangeney N, Niel P, Paul G et al. A 5-year epidemiological study of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae isolates in a medium- and long-stay neurological unit. *J Appl Microbiol.* 2000;88:504-511.

(67) Lucet JC, Chevret S, Decre D et al. Outbreak of multiply resistant enterobacteriaceae in an intensive care unit: epidemiology and risk factors for acquisition. *Clin Infect Dis.* 1996;22:430-436.

(68) De CC, Rouby D, Guelon D et al. A case-control study of an outbreak of infections caused by Klebsiella pneumoniae strains producing CTX-1 (TEM-3) beta-lactamase. *J Hosp Infect.* 1991;18:5-13.

(69) Ho PL, Chan WM, Tsang KW, Wong SS, Young K. Bacteremia caused by Escherichia coli producing extended-spectrum beta-lactamase: a case-control study of risk factors and outcomes. *Scand J Infect Dis.* 2002;34:567-573.

(70) Menashe G, Borer A, Yagupsky P et al. Clinical significance and impact on mortality of extended-spectrum beta lactamase-producing Enterobacteriaceae isolates in nosocomial bacteremia. *Scand J Infect Dis.* 2001;33:188-193.

(71) Piroth L, Aube H, Doise JM, Vincent-Martin M. Spread of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae: are beta-lactamase inhibitors of therapeutic value? *Clin Infect Dis.* 1998;27:76-80.

(72) D'Agata E, Venkataraman L, DeGirolami P, Weigel L, Samore M, Tenover F. The molecular and clinical epidemiology of enterobacteriaceae-producing extended-spectrum beta-lactamase in a tertiary care hospital. *J Infect.* 1998;36:279-285.

(73) Kim YK, Pai H, Lee HJ et al. Bloodstream infections by extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in children: epidemiology and clinical outcome. *Antimicrob Agents Chemother.* 2002;46:1481-1491.

(74) Lin MF, Huang ML, Lai SH. Risk factors in the acquisition of extended-spectrum beta-lactamase Klebsiella pneumoniae: a case-control study in a district teaching hospital in Taiwan. *J Hosp Infect.* 2003;53:39-45.

(75) Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for infection and impact of resistance on outcomes. *Clin Infect Dis.* 2001;32:1162-1171.

(76) Revathi G SS. Detection of Expanded spectrum cephalosporin resistance due to inducible lactamases in hospital isolates. *Indian J Med Microbiol 1997.* 1997;113-115.

(77) Emery CL, Weymouth LA. Detection and clinical significance of extended-spectrum beta-lactamases in a tertiary-care medical center. *J Clin Microbiol.* 1997;35:2061-2067.

(78) Clinical and Laboratory Standards Institute. and Document M100-S16. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement. http://wwwn.cdc.gov/nltn/pdf/2006/3_M100-S16_checklist_D2.pdf . 2006.
Ref Type: Electronic Citation

(79) Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev Infect Dis.* 1988;10:867-878.

(80) Thomson KS, Sanders CC. Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests. *Antimicrob Agents Chemother.* 1992;36:1877-1882.

(81) Ho PL, Chow KH, Yuen KY, Ng WS, Chau PY. Comparison of a novel, inhibitor-potentiated disc-diffusion test with other methods for the detection of extended-spectrum beta-lactamases in Escherichia coli and Klebsiella pneumoniae. *J Antimicrob Chemother.* 1998;42:49-54.

(82) Sanders CC, Barry AL, Washington JA et al. Detection of extended-spectrum-beta-lactamase-producing members of the family Enterobacteriaceae with Vitek ESBL test. *J Clin Microbiol.* 1996;34:2997-3001.

(83) Vercauteren E, Descheemaeker P, Ieven M, Sanders CC, Goossens H. Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among blood isolates of Escherichia coli and Klebsiella spp. in a Belgian teaching hospital. *J Clin Microbiol.* 1997;35:2191-2197.

(84) Tenover FC, Raney PM, Williams PP et al. Evaluation of the NCCLS extended-spectrum beta-lactamase confirmation methods for Escherichia coli with isolates collected during Project ICARE. *J Clin Microbiol.* 2003;41:3142-3146.

(85) Kader AA, Angamuthu KK, Kamath KA, Zaman MN. Modified double-disc test for detection of extended-spectrum beta-lactamases in Escherichia coli and Klebsiella pneumoniae. *Br J Biomed Sci.* 2006;63:51-54.

(86) Coudron PE, Moland ES, Sanders CC. Occurrence and detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae at a veterans medical center: seek and you may find. *J Clin Microbiol.* 1997;35:2593-2597.

(87) Moland ES, Thomson KS. Extended-spectrum beta-lactamases of Enterobacteriaceae. *J Antimicrob Chemother.* 1994;33:666-668.

(88) Mendonca N, Ferreira E, Canica M. Occurrence of a novel SHV-type enzyme (SHV-55) among isolates of Klebsiella pneumoniae from Portuguese origin in a comparison study for extended-spectrum beta-lactamase-producing evaluation. *Diagn Microbiol Infect Dis.* 2006;56:415-420.

(89) Sirot D. Extended-spectrum plasmid-mediated beta-lactamases. *J Antimicrob Chemother.* 1995;36 Suppl A:19-34.:19-34.

(90) Rice LB, Willey SH, Papanicolaou GA et al. Outbreak of ceftazidime resistance caused by extended-spectrum beta-lactamases at a Massachusetts chronic-care facility. *Antimicrob Agents Chemother.* 1990;34:2193-2199.

(91) Meyer KS, Urban C, Eagan JA, Berger BJ, Rahal JJ. Nosocomial outbreak of Klebsiella infection resistant to late-generation cephalosporins. *Ann Intern Med.* 1993;119:353-358.

(92) Lucet JC, Decre D, Fichelle A et al. Control of a prolonged outbreak of extended-spectrum beta-lactamase-producing enterobacteriaceae in a university hospital. *Clin Infect Dis.* 1999;29:1411-1418.

(93) Rice LB, Eckstein EC, DeVente J, Shlaes DM. Ceftazidime-resistant Klebsiella pneumoniae isolates recovered at the Cleveland Department of Veterans Affairs Medical Center. *Clin Infect Dis.* 1996;23:118-124.

(94) Mebis J, Goossens H, Bruyneel P et al. Decreasing antibiotic resistance of Enterobacteriaceae by introducing a new antibiotic combination therapy for neutropenic fever patients. *Leukemia.* 1998;12:1627-1629.

(95) Ahmad M, Urban C, Mariano N et al. Clinical characteristics and molecular epidemiology associated with imipenem-resistant Klebsiella pneumoniae. *Clin Infect Dis.* 1999;29:352-355.

(96) Go ES, Urban C, Burns J et al. Clinical and molecular epidemiology of acinetobacter infections sensitive only to polymyxin B and sulbactam. *Lancet.* 1994;344:1329-1332.

(97) Shanmuganathan C, Ananthakrishnan A, Jayakeerthi SR et al. Learning from an outbreak: ESBL- the essential points. *Indian J Med Microbiol.* 2004;22:255-257.

(98) Chow JW, Fine MJ, Shlaes DM et al. Enterobacter bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med.* 1991;115:585-590.

(99) Chow JW, Yu VL. Combination antibiotic therapy versus monotherapy for gram-negative bacteraemia: a commentary. *Int J Antimicrob Agents.* 1999;11:7-12.

(100) Ratanarat R, Thanakittiwirun M, Vilaichone W, Thongyoo S, Permpikul C. Prediction of mortality by using the standard scoring systems in a medical intensive care unit in Thailand. *J Med Assoc Thai.* 2005;88:949-955.

(101) Handschu R, Haslbeck M, Hartmann A et al. Mortality prediction in critical care for acute stroke: Severity of illness-score or coma-scale? *J Neurol.* 2005;252:1249-1254.

(102) Le G, Jr., Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA.* 1993;270:2957-2963.

(103) Chow JW, Fine MJ, Shlaes DM et al. Enterobacter bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med.* 1991;115:585-590.

(104) Rahal JJ, Urban C, Horn D et al. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial Klebsiella. *JAMA.* 1998;280:1233-1237.

(105) Gross PA, Barrett TL, Dellinger EP et al. Quality standard for the treatment of bacteremia. Infectious Diseases Society of America. *Clin Infect Dis.* 1994;18:428-430.

APPENDIX 1

PITTS s bacteremia score :[36,99]

Fever (oral temperature)	
≤35°C or ≥40°C	2
35.1–36.0°C or 39.0–39.9°C	1
36.1–38.9°C	0
Hypotension	2
Acute hypotensive event with drop in systolic blood pressure > 30 mm Hg and diastolic blood pressure > 20 mm Hg or Requirement for intravenous vasopressor agents or Systolic blood pressure < 90 mm Hg	
Mechanical ventilation	2
Cardiac arrest	4
Mental status	
Alert	0
Disoriented	1
Stuporous	2
Comatose	4

APPENDIX 2

SAPS II scoring system :

Variables		Points
Type of admission	Unscheduled surgery	8
	Medical	6
	Scheduled surgery	0
Chronic diseases	None	0
	Metastasic Carcinoma	9
	Hematological malignancy	10
	AIDS	17
Glasgow coma scale	<6	26
	6-8	13
	9-10	7
	11-13	5
	14-15	0
Age	<40	0
	40-59	7
	50-59	12
	60-69	15
	70-79	17
	>80	18
Systolic BP-mm Hg	<70	13
	70-99	5
	100-199	0
	>200	2
Heart Rate	<40	11
	40-69	2
	70-119	0
	120-159	4
	>160	7
Temperature ºC	<39	0
	>39	3
PaO_2 /FiO_2	<100	11
	100-199	9
	>200	6
Urine output /24 hrs in ml	<500	11
	500-999	4
	>1000	0
Serum Urea mg/dL	<28	0
	28-83	6
	>84	10

WBC /mm^3	<1000	12
	1000-19000	0
	>19000	3
Serum Potassium mEq/L	<3	3
	3-4.5	0
	>4.5	3
Serum Sodium mEq/L	>145	1
	125-144	0
	<124	5
Serum Bicarbonate mEq/L	<15	6
	15-19	3
	>20	0
Serum Bilirubin mg/dL	<4	0
	4-5.9	4
	>6	9

SAPS II score calculation; the total score is calculated from the above points for each variable.

The predicted mortality rate is calculated from the following equation

Logit = -7,7631+0,0737* (SAPSII) + 0,9971* ln ((SAPSII)+1)

Predicted Death Rate =$e^{(Logit)}/(1+e^{(Logit)})$

Definitions for SAPS score:

Age: Use the patient's age in years at last birthday.

Heart rate: Use the worst value in 24 hours, either low or high rate; if it varied from cardiac arrest (11pts) to extreme tachycardia (7pts), assign 11points.

SBP: Use the same method as for hearth rate: eg, if it varied from 60 mmHg to 205mmHg, assign 13 points.

Body temperature: Use the highest temperature in °C or °F.

PaO2/FiO2 ratio: If ventilated or CPAP, use the lowest value of the ratio.

Urinary output: If the patient is in the intensive care unit for less than 24 hours, make the calculation for 24 hours.

Serum Urea or BUN: Use the highest value in mmol// or g/L for serum urea, in mg/dL for the serum urea nitrogen.

WBC count: Use the worst (high or low) WBC count.

Serum potassium level: Use the worst (high or low) value.

Serum Sodium level: Use the worst (high or low) value.

Serum bicarbonate level: Use the lowest value.

Bilirubin : Use the highest value in micromol/L or mg/dL.

Glasgow Coma Score (GCS) : Use the lowest value. If the patient is sedated, record the estimated GCS before sedation.

AIDS: Yes, if HIV positive with clinical complications as *Pneumocystis carinii* pneumonia, Kaposi's sarcoma, Lymphoma, tuberculosis or toxoplasma infection.

Hematologic malignancy: Yes, if lymphoma, acute leukemia, or multiple myeloma.

Metastatic cancer: Yes, if proven metastasis by surgery, C.T. scan or any other method.

APPENDIX 3

PROFORMA

Name

Age :

Sex :

Address :

Phone number:

Hospital No. :

Ward : ICU/ non ICU

Department :

Date of admission :

Date of isolation of organism :

Date of discharge:

Organism grown on culture: Klebsiella / *E.coli*

ESBL production : Yes / No

Primary diagnosis :

Co morbidities :

- diabetes mellitus
- hypertension
- chronic liver disease
- chronic renal failure
- ischemic heart disease
- hematological malignancy
- solid tumor
- HIV- AIDS

Probable source of infection :

- gastrointestinal
- genitourinary
- soft tissue
- pneumonia
- CNS infection
- primary blood stream infection
- others
- undetermined

Prior antibiotic use :

- Penicillin
- Cephalosporin
- Quinolones
- Aminoglycosides
- Carbapenems
- Others

Initial antibiotic used:

- Cefotaxime
- Ceftazidime
- Ciprofloxacin
- Gentamicin
- Netilmicin
- Amikacin
- Piptaz
- Ticarcillin/Clavulanate
- Cefoperazone/Sulbactam
- Imipenem
- Meropenem

Culture sensitivity :

Antibiotic	S	I	R
Cefotaxime			
Ceftazidime			
Ciprofloxacin			
Gentamicin			
Netilmicin			
Amikacin			
Piptaz			
Ticarcillin/Clav			
Cefoperazone/Sul			
Imipenem			
Meropenem			

2 week follow up :

- Alive
- Dead
- Lost to follow up

PITTS score

SAPS II score

APPENDIX 4

INFORMED CONSENT FORM

I, Mr / Mrs ……..,Hosp No…….. son/daughter/wife of Mr…….. , from .....,have been explained in detail about the proposed study of epidemiology of 2 bacteria (*Escherichia coli* and Klebsiella)causing blood stream infection. I understand that no intervention will be done as part of the study and treatment would depend entirely on the treating physician and will not be influenced by the study .I hereby give consent to be part of the proposed study, and am willing to be under follow up for 2 weeks. I also understand that I can withdraw from the study at any time.

Signature of the patient/relative

Signature of the doctor

5. Glossary for master sheet

Dept - Department
DOD - Date of admission
DOD - Date of discharge
DOI - Date of isolation of organism
Durn - Duration of stay
Noso/ Com- Nosocomial / community acquired
HTN - Hypertension
Solid - Solid tumors
Hemat - Hamtological Malignancy
Surgery - Recent surgery
CLD - Chronic Liver Disease
DM - Diabetes Mellitus
IHd - Ischemic Heart Disease
CRF - Chronic Renal Failure
Source
1 - urinary tract
2- intra abdominal
3 - undetermined
4- pneumonia
Priorant - Prior antibiotic
prBeta - Prior beta lactam
prcef - Prior cephalosporins
pramin - Prior aminoglycosides
prquin - Prior Quinolones
prcarb - Prior carbapenems
prothers - Prior other antibiotics
incefo - Initial antibiotic used was Cefotaxime
incefta - Initial antibiotic used was Ceftazidime
incefep - Initial antibiotic used was Cefepime
incefoper - Initial antibiotic used was Cefoperazone - sulbactam
incipro- Initial antibiotic used was Ciprofloxacin
inoquin - Initial antibiotic used were quinolones other than Ciprofloxacin
ingenta- Initial antibiotic used was Gentamicin
inamin - Initial antibiotic used was Amikacin
innet - Initial antibiotic used was Netilmicin
inpip - Initial antibiotic used was Piperacillin - Tazobactam
intic - Initial antibiotic used was Ticarcillin
inimi - Initial antibiotic used was Imipenem
inmero - Initial antibiotic used was Meropenem
inaug - Initial antibiotic used was Augmentin
inothers - Others used as initial therapy
approp - whether initial antibiotic was appropriate
ESBL - ESBL production
Predmort - Predicted mortality

Name	Hospital I	Age	Sex	Dept	DOA	DOD	DOI	Dur	Organism	Noso/Com	Diagnosis	HTN	Solid
Parimala	446615b	48	female	Medicine	07.02.07	21.02.07	08.02.07	15	E coli	Nosocomial	Urosepsis	No	no
Jegajothi	973142c	60	female	Medicine	09.02.07	08.03.07	23.02.07	28	E coli	Nosocomial	Disseminated malignancy	No	Yes
Bakthinathan	512907c	56	male	Medicine	15.04.07	05.05.07	15.04.07	21	E coli	Nosocomial	Urosepsis	Yes	no
Latha	999047c	32	female	Medicine	31.03.07	14.04.07	05.04.07	15	E coli	Nosocomial	Urosepsis with DKA	No	no
Arati bhattacha	021528d	64	female	Medicine	04.05.07	20.05.07	10.05.07	17	E coli	Nosocomial	PUO	No	no
Lakshmana	017027d	45	male	Medicine	26.04.07	06.06.07	16.05.07	42	E coli	Nosocomial	Acute Pancreatitis	Yes	no
Desai mavi	354269c	48	male	Medicine	19.05.07	21.05.07	17.05.07	3	E coli	Nosocomial	Pleural effusion	Yes	no
Nizamudeen	972917c	63	male	Hematology	02.02.07	10.03.07	21.02.07	37	E coli	Nosocomial	AML	No	no
Dipu Malakar	988782c	34	male	Hematology	03.04.07	23.04.07	19.04.07	21	E coli	Nosocomial	AML	No	no
Ganesan	884281c	52	male	GEC	25.01.07	15.02.07	06.02.07	22	E coli	Nosocomial	DCLD-Cryptogenic	No	no
Hannah	012841d	49	female	other medical	21.04.07	07.05.07	21.04.07	17	E coli	Nosocomial	Pyelonephritis	No	no
Swapna Ghosh	984162c	54	female	other medical	12.04.07	05.05.07	22.04.07	24	E coli	Nosocomial	Diabetic neuropathy	No	no
Rama Devi	967463c	44	female	Other surgical	24.02.07	03.04.07	28.02.07	39	E coli	Nosocomial	Mucinous cystadenoma ovary	Yes	no
Ranjan kumar	970690c	21	male	Hematology	30.01.07	21.02.07	04.02.07	22	Klebsiella	Nosocomial	Pre B ALL	No	no
Subramaniam	030681d	60	male	Hematology	22.05.06	01.06.07	27.05.06	11	Klebsiella	Nosocomial	ALL	No	no
Taharur	025799d	27	female	other medical	17.05.07	11.06.07	30.05.07	26	Klebsiella	Nosocomial	TEN	No	no
Shyam Sundar	974900c	51	male	Other surgical	25.04.07	11.05.07	01.05.07	17	Klebsiella	Nosocomial	Voluntary Kidney donor	No	no
Venugopal	965733c	48	male	Other surgical	27.01.07	08.02.07	03.02.07	13	Klebsiella	Nosocomial	RTA	No	no
Siba Shankar	997294c	69	male	ICU	23.03.07	31.05.07	13.05.07	70	Klebsiella	Nosocomial	PPI Endocarditis	No	no
Loganathan	993167c	46	male	Medicine	19.03.07	09.04.07	19.03.07	22	E coli	Community	Rt Pyelonephritis	No	no
Gnanamani	985754c	34	female	Medicine	23.03.07	25.03.07	24.03.07	3	E coli	Community	Pyelonephritis	No	no
Palani	029842d	37	male	Other surgical	26.05.07	27.05.07	24.05.07	2	E coli	Community	Pyelonephritis	No	no
Narayana chowd	896143c	60	male	Medicine	15.02.07	30.03.07	16.03.07	44	E coli	Nosocomial	Craniopharyngioma	Yes	Yes
Krishna	884539c	28	male	Medicine	04.04.07	26.04.07	10.04.07	23	E coli	Nosocomial	Sepsis	No	no
Srinivasulu	808349c	76	male	Surgery	24.04.07	07.05.07	02.05.07	14	E coli	Nosocomial	laproscopic Cholecystectomy	No	no
Rajan	430288c	40	male	Hematology	13.04.07	03.05.07	03.05.07	21	E coli	Nosocomial	Aplastic anemia	No	no
Pushpa Giri	963898c	41	male	Hematology	19.01.07	13.03.07	01.03.07	53	E coli	Nosocomial	AML	No	no
Rajat	981651c	27	male	Hematology	04.05.07	31.05.07	19.05.07	28	E coli	Nosocomial	ALL	No	no
Chandrasekhar	025503d	62	male	Other surgical	11.05.07	17.05.07	11.05.07	7	E coli	Nosocomial	Pyelonephritis	Yes	no
Manoj Kumar	970366c	22	male	Other surgical	10.03.07	30.03.07	27.03.07	21	E coli	Nosocomial	Ameloblastoma	No	no
Ponkodi	013460d	23	female	Medicine	26.04.07	11.05.07	11.05.07	16	Klebsiella	Nosocomial	DKA	No	no
Alphy Kurien	942535c	21	male	Hematology	23.01.07	13.04.07	03.03.07	81	Klebsiella	Nosocomial	pre B ALL	No	no
Tafsil Begum	902665c	30	female	Hematology	05.02.07	30.04.07	22.03.07	85	Klebsiella	Nosocomial	AML	No	no
Ramalingam	957367c	76	male	Medicine	23.04.07	08.05.07	23.04.07	16	E coli	Community	Urosepsis	No	no
Srinivasulu	018385d	51	male	Medicine	04.05.07	07.05.07	04.05.07	4	E coli	Community	Urosepsis	No	no
Victoria	896462	38	female	Surgery	29.04.07	16.05.07	30.04.07	18	E coli	Community	Pyelonephritis	No	no

Ponnuswamy	463128c	65	male	Hematology	30.04.07	01.05.07	30.04.07	2	E coli	Community	Multiple Myeloma	Yes	no
Labubala	988622c	55	female	Other surgical	30.03.07	11.04.07	30.03.07	13	E coli	Nosocomial	CABG post op	No	no
Mirunalini	988205c	52	female	ICU	09.03.07	25.03.07	11.03.07	17	E coli	Nosocomial	PUO	No	no
Rajendran	978708c	55	male	ICU	31.03.07	09.04.07	01.04.07	10	E coli	Nosocomial	Cellulitis Rt Gluteal region	No	no
Tarun Bhowmik	989790c	45	male	Surgery	13.05.07	08.06.07	21.05.07	27	Klebsiella	Nosocomial	Familial adenomatous polypos	No	no
Khadar	967925c	66	male	Hematology	05.02.07	23.02.07	13.02.07	19	Klebsiella	Nosocomial	Aplastic anemia	No	no
Murthy	990073c	46	male	Medicine	10.03.07	21.03.07	11.03.07	22	E coli	Nosocomial	Urosepsis	No	no
Kodandan	035024d	75	male	Medicine	28.05.07	08.06.07	28.05.07	2	E coli	Nosocomial	Urosepsis	No	no
Hillarius	953800c	65	male	Surgery	12.02.07	06.03.07	18.02.07	23	E coli	Nosocomial	Periampullary carcinoma	Yes	Yes
Rajeswari	977168c	50	female	Hematology	16.02.07	28.04.07	27.02.07	72	E coli	Nosocomial	AML	No	no
Mohammed Nas	982892c	49	male	GEC	15.03.07	09.04.07	30.03.07	25	E coli	Nosocomial	DCLD -HCV related,HCC	No	Yes
Shankari Shili	734648c	31	female	other medical	14.04.07	18.04.07	17.04.07	5	E coli	Nosocomial	Nephrotic syndrome	Yes	no
Sivaraj	013922d	51	male	other medical	21.04.07	07.05.07	24.04.07	17	E coli	Nosocomial	NSTEMI	Yes	no
Badal maity	980035c	51	male	other medical	27.02.07	08.03.07	28.02.07	10	E coli	Nosocomial	Urosepsis	No	no
Suraiya	705095c	58	female	other medical	16.05.07	05.06.07	30.05.07	21	E coli	Nosocomial	CKD	Yes	no
Thanigavathy	997937c	26	female	Other surgical	30.03.07	03.04.07	31.03.07	5	E coli	Nosocomial	Postpartum	No	no
Sandhya Shaw	587451c	45	female	GEC	16.04.07	01.06.07	17.05.07	47	Klebsiella	Nosocomial	PUO	No	no
Surendra	999010c	47	male	ICU	27.03.07	19.04.07	01.04.07	24	Klebsiella	Nosocomial	RTA	No	no
Dhakshinamurth	880731c	63	male	Medicine	22.03.07	30.03.07	21.03.07	9	E coli	Community	Urosepsis	No	no
Krishnamurthy	959021c	78	male	Medicine	06.02.07	20.02.07	06.02.07	15	E coli	Community	Urosepsis	No	no
Kesavan	919636c	76	male	Medicine	30.03.07	16.04.07	31.03.07	18	E coli	Community	Urosepsis	Yes	no
Ravi E	999428c	46	male	Medicine	06.04.07	20.04.07	06.04.07	15	E coli	Community	DKA	No	no
Salammal	339662c	63	female	Medicine	01.05.07	02.05.07	02.05.07	2	E coli	Community	Urosepsis	Yes	no
Deivanai	971476c	75	female	Medicine	12.02.07	12.02.07	12.02.07	1	E coli	Community	Urosepsis	No	no
Mary Philip	051571c	82	female	Medicine	18.04.07	19.04.07	19.04.07	2	E coli	Community	LRI	No	no
Manoj Biswas	013790d	39	male	GEC	08.05.07	14.05.07	08.05.07	7	E coli	Community	DCLD -HCV related	No	no
Murugamma	006611d	54	female	Other surgical	10.04.07	19.04.07	10.04.07	10	E coli	Community	Pyelonephritis emphysematou	No	no
Ponkodi	013460d	23	female	ICU	28.04.07	11.05.07	25.04.07	14	E coli	Community	Urosepsis	No	no
Kanniappan	758289a	71	male	Medicine	29.03.07	30.03.07	29.03.07	2	Klebsiella	Community	Bronchiectasis	Yes	no
Shanmugavel	002344d	64	male	GEC	30.03.07	02.04.07	30.03.07	4	Klebsiella	Community	Fulminant hepatitis E	No	no
Periyasamy	965958c	40	male	Medicine	01.02.07	20.02.07	01.02.07	20	E coli	Nosocomial	Urosepsis	No	no
Sampoornam	030561d	54	female	Medicine	25.05.06	07.06.07	25.05.06	14	E coli	Nosocomial	Urosepsis	No	no
Thiruvanakarasu	025544d	39	male	Medicine	12.05.07	21.05.07	18.05.07	10	E coli	Nosocomial	Aseptic Meningitis	No	no
Sethuraman	993224c	26	male	Medicine	20.03.07	10.04.07	30.03.07	22	E coli	Nosocomial	OP poisoning	No	no
Soundari	972817c	20	female	Surgery	04.02.07	15.02.07	04.02.07	12	E coli	Nosocomial	TB ileal perforation	No	no
Ravanth Kumar	023646d	20	male	Surgery	10.05.07	19.05.07	13.05.07	10	E coli	Nosocomial	RTA	No	no
Jain D	027237d	54	male	Surgery	15.05.07	21.05.07	19.05.07	7	E coli	Nosocomial	Cheledocholithiasis	No	no

Krishnan	993401c	55	male	Surgery	25.03.07	08.04.07	26.03.07	15	E coli	Nosocomial	Necrotising fasciitis Lt LL	No	no
Varghese	960895c	70	male	Hematology	22.02.07	14.03.07	02.03.07	21	E coli	Nosocomial	B cell Lymphoma	Yes	Yes
Hajee Md	726044c	54	male	Hematology	03.04.07	16.04.07	15.04.07	14	E coli	Nosocomial	Metastatic neuroendocrine tun	No	Yes
Ramesh Chandr	988823c	60	male	GEC	08.03.07	14.03.07	09.03.07	7	E coli	Nosocomial	DCLD-HBV related	No	no
Narmada	991457c	59	female	other medical	22.04.07	09.05.07	22.04.07	18	E coli	Nosocomial	Bronchogenic carcinoma	No	Yes
Rev Paul Thoma	215605c	66	male	other medical	22.01.07	10.04.07	11.03.07	79	E coli	Nosocomial	Demyelinating disease	Yes	no
Saravanan	013422d	26	male	other medical	28.04.07	07.05.07	29.04.07	11	E coli	Nosocomial	Pyelonephritis	No	no
Shameen	970470c	41	female	Other surgical	29.01.07	10.02.07	05.02.07	13	E coli	Nosocomial	Post partum Febrile illness	Yes	no
Chinnakilli	908241c	63	female	Other surgical	17.05.07	10.06.07	19.05.07	25	E coli	Nosocomial	CABG post op	Yes	no
Naganathan	965860c	84	male	Surgery	30.01.07	03.02.07	01.02.07	5	Klebsiella	Nosocomial	Obstr periumbilical hernia	No	no
Rita Rani	210983c	49	female	Surgery	12.01.07	21.04.07	13.02.07	##	Klebsiella	Nosocomial	Ulcerative colitis	No	no
Bhupathy	019208d	30	male	Hematology	29.04.07	06.05.07	05.05.07	8	Klebsiella	Nosocomial	AML	No	no
Rama Kanta Jar	936554c	51	male	other medical	06.02.07	09.04.07	23.03.07	63	Klebsiella	Nosocomial	ADPKD	Yes	no
Jayakumar	753923c	36	male	ICU	07.05.07	08.05.07	06.05.07	2	Klebsiella	Nosocomial	Acute Pancreatitis	No	no
Fathima	641040b	59	female	ICU	23.03.07	08.04.07	23.03.07	14	Klebsiella	Nosocomial	Aspiration pneumonia	Yes	no
Sekar	006839d	45	male	Medicine	13.04.07	02.05.07	14.04.07	20	E coli	Community	Urosepsis	No	no
Kanchana	287037a	67	female	Medicine	17.04.07	03.05.07	17.04.07	17	E coli	Community	Urosepsis	Yes	no
Anjali Saha	014040d	50	female	Surgery	25.04.07	07.05.07	25.04.07	13	E coli	Community	Cholangitis	No	no
Mahadevan	013853a	50	male	other medical	11.05.07	13.05.07	12.05.07	4	E coli	Community	Lupus nephritis	No	no
Thavamani	993255c	45	female	Surgery	27.04.07	30.04.07	27.04.07	4	E coli	Nosocomial	Urosepsis	No	no
Varalaxmi	013202d	20	female	Medicine	22.04.07	04.05.07	23.04.07	13	E coli	Community	Urosepsis	No	no
Shenbagaraj	968704c	53	male	GEC	14.02.07	19.02.07	14.02.07	6	E coli	Community	CLD	No	no
Pradeep Peter	986326c	23	male	GEC	18.04.07	20.04.07	19.04.07	3	E coli	Community	DCLD	No	no
Salamma	988269c	49	female	Medicine	11.03.07	17.03.07	13.03.07	7	E coli	Nosocomial	Cerebrovascular accident	Yes	no
Vijaya Kennedy	783309c	38	female	Hematology	17.03.07	19.04.07	30.03.07	33	E coli	Nosocomial	CML	No	no
Washington	383868b	51	male	GEC	20.04.07	01.05.07	24.04.07	12	Klebsiella	Nosocomial	DCLD- Ethanol related	No	no
Jothi	114676c	70	female	Medicine	19.03.07	31.03.07	20.03.07	13	E coli	Community	Cerebrovascular accident	Yes	no
Rugmani	432911b	55	female	Medicine	30.04.07	05.05.07	30.04.07	6	E coli	Community	Pyelonephritis	No	no
Valliyammal	685782c	73	female	Medicine	07.05.07	09.05.07	07.05.07	3	E coli	Community	Urosepsis	Yes	no
Vadivambal	975700c	64	female	Medicine	16.02.07	22.02.07	16.02.07	7	E coli	Community	Acute coronary syn	Yes	no
Balaji	029999d	25	male	GEC	28.05.07	05.06.07	28.05.07	9	E coli	Community	Wilsons disease	No	no
Logaranjan	744329c	37	male	GEC	10.05.07	16.05.07	11.05.07	7	E coli	Community	DCLD -Ethanol related	No	no
Sheik Nawab	013204d	39	male	GEC	22.04.07	23.04.07	23.04.07	2	E coli	Community	Subacute hepatic failure	No	no
Rajamma	671792c	70	female	other medical	25.03.07	29.03.07	25.03.07	5	E coli	Community	Urosepsis	Yes	no
Saroja	324268b	68	female	other medical	25.05.07	29.05.07	26.05.07	5	E coli	Community	Pyelonephritis	Yes	no
Devaraj	029784d	37	male	ICU	22.05.07	03.06.07	22.05.07	13	E coli	Community	CLD - Alcohol related	No	no
Purnima Ghosh	027584d	39	female	GEC	17.05.07	22.05.07	16.05.07	6	Klebsiella	Community	Cholangitis	No	no

Thakur	008003d	50	male	ICU	10.04.07	12.04.07	10.04.07	3	Klebsiella	Community	Exudative pericarditis	No	no
Rabindra	001593d	47	male	GEC	14.04.07	18.04.07	16.04.07	5	E coli	Nosocomial	DCLD	No	no
Toshi Ao	003069d	35	male	GEC	04.04.07	15.04.07	12.04.07	12	E coli	Nosocomial	Disseminated malignancy	No	Yes
Sandha	933942c	58	female	other medical	09.02.07	12.02.07	08.02.07	4	E coli	Nosocomial	Carcinoma Cervix	No	no
Sabtri Shaw	992797c	42	female	Surgery	07.05.07	22.05.07	14.05.07	16	Klebsiella	Nosocomial	Carcinoma head of pancreas	No	Yes
Devakumari	975777c	48	female	Medicine	28.02.07	03.03.07	28.02.07	4	E coli	Community	Urosepsis	No	no
Kamala bai	486808a	89	female	Medicine	23.03.07	09.04.07	24.03.07	15	E coli	Community	Urosepsis	Yes	no
Thirumala reddy	993038c	60	male	Medicine	15.03.07	28.03.07	15.03.07	14	E coli	Community	Sepsis	No	no
Gopuram Ravi	986527c	39	male	Medicine	28.02.07	12.03.07	01.03.07	13	E coli	Community	Chronic pancreatitis	No	no
Adeline Priya	641312c	23	female	Hematology	30.03.07	16.04.07	30.03.07	18	E coli	Community	AML	No	no
Sahajada Md	336481c	30	male	Hematology	18.03.07	18.03.07	18.03.07	1	E coli	Community	Aplastic anemia	No	no
Bhaskar	704754c	46	male	GEC	12.03.07	11.04.07	12.03.07	31	E coli	Community	CLD -Ethanol related	No	no
Rubini	937919c	22	female	Other surgical	06.04.07	14.04.07	06.04.07	9	E coli	Community	Postpartum urosepsis	No	no
Dhanuram	972816c	57	male	ICU	02.02.07	10.02.07	05.02.07	9	E coli	Nosocomial	CRF	No	no
Prakash	948090b	33	male	Hematology	05.05.07	06.06.07	14.05.07	33	Klebsiella	Nosocomial	CML	No	no
Vijayalakshmi	801826c	33	female	other medical	20.05.07	21.05.07	01.05.07	2	E coli	Community	Renal allograft recipient	No	no
Nagaraju	910556c	23	male	Hematology	04.02.07	13.02.07	04.02.07	10	E coli	Community	AML	No	no
Thangavel	006698d	68	male	Hematology	13.04.07	04.05.07	13.04.07	22	Klebsiella	Community	B cell Lymphoma	No	no
Kananath	963069c	45	male	Surgery	19.03.07	02.04.07	31.03.07	14	Klebsiella	Nosocomial	Periampullary carcinoma	No	Yes
Rani Selvaraj	642219a	38	female	Surgery	17.02.07	05.05.07	19.04.07	78	Klebsiella	Nosocomial	Chronic pancreatitis	No	no
Chitra	971462c	43	female	other medical	05.03.07	13.03.07	06.03.07	9	E coli	Nosocomial	Renal Calculus	No	no

Hemat	Surgery	CLD	DM	HIV	IHD	CRF	Source	Priorant	Prbeta	Prcef	Pramin	Prquin	Prcarb	Prothers	Incefot	Incefta	Incefep	Incefoper	Incipro
No	No	No	Yes	No	No	No	1	No	No	No	No	No	No	No	No	No	No	No	No
No	No	No	No	No	No	No	1	yes	No	No	No	Yes	No	No	No	No	No	No	No
No	No	No	Yes	No	Yes	No	1	yes	No	Yes	No	No	No	No	No	No	No	No	No
No	No	No	Yes	No	No	No	1	yes	No	Yes	Yes	No	No	No	No	No	No	No	No
No	No	No	Yes	No	No	No	3	No	No	No	No	No	No	No	No	No	No	No	No
No	No	Yes	Yes	No	No	No	2	yes	No	Yes	No	No	No	No	No	No	No	No	No
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Yes	No	No	Yes	No	No	No	3	yes	No	No	No	Yes	No	Yes	No	No	No	No	No
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No	No	Yes	Yes	No	No	No	2	yes	No	Yes	No	Yes	No	No	No	No	No	No	No
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No	Yes	No	No	No	No	No	2	yes	No	Yes	Yes	No	No	Yes	No	Yes	No	Yes	No
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Yes	No	No	No	No	No	No	4	No	No	No	No	No	No	No	Yes	No	No	No	No
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No	No	No	Yes	No	No	No	3	No	No	No	No	No	No	No	No	No	No	No	No
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No	No	Yes	No	No	No	No	2	No	No	No	No	No	No	No	Yes	No	No	No	No
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Yes	No	No	No	No	No	No	3	yes	No	No	No	Yes	No	No	No	No	Yes	No	No
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No	No	Yes	No	No	No	No	2	No	No	No	No	No	No	No	Yes	No	No	No	No
No	No	Yes	Yes	No	No	No	2	No	No	No	No	No	No	No	No	No	Yes	No	No
No	No	No	Yes	No	No	No	1	No	No	No	No	No	No	No	Yes	No	No	No	No
No	No	No	Yes	No	No	No	1	No	No	No	No	No	No	No	No	Yes	No	No	No
No	No	Yes	No	No	No	No	2	No	No	No	No	No	No	No	No	No	Yes	No	No
No	Yes	No	Yes	No	No	No	2	No	No	No	No	No	No	No	No	Yes	No	No	No

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No	No	Yes	No	No	No	No	2	No	No	No	No	No	No	No	Yes	No	No	No	No
No	No	No	No	No	No	No	3	No	No	No	No	No	No	No	No	No	No	No	No
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No	Yes	No	No	No	No	No	2	yes	No	Yes	No	No	No	No	Yes	No	No	No	No
No	No	No	No	No	No	No	1	No	No	No	No	No	No	No	Yes	No	No	No	Yes
No	No	No	No	No	Yes	No	1	No	No	No	No	No	No	No	Yes	No	No	No	Yes
No	No	No	No	No	No	No	3	No	No	No	No	No	No	No	Yes	No	No	No	No
No	No	No	No	Yes	No	No	2	No	No	No	No	No	No	No	No	No	Yes	No	No
Yes	No	No	No	No	No	No	3	No	No	No	No	No	No	No	No	No	Yes	No	No
Yes	No	No	No	No	No	No	3	No	No	No	No	No	No	No	No	No	No	No	No
No	No	Yes	No	No	No	No	1	No	No	No	No	No	No	No	Yes	No	No	No	No
No	No	No	No	No	No	No	1	No	No	No	No	No	No	No	No	No	No	No	No
No	No	No	Yes	No	Yes	Yes	1	No	No	No	No	No	No	No	No	No	Yes	No	No
Yes	No	No	No	No	No	No	3	No	No	No	No	No	No	No	No	No	Yes	No	No
No	No	No	No	No	No	Yes	1	No	No	No	No	No	No	No	No	No	Yes	No	No
Yes	No	No	No	No	No	No	3	No	No	No	No	No	No	No	Yes	No	No	No	No
Yes	Yes	No	No	No	No	No	3	No	No	No	No	No	No	No	Yes	No	Yes	No	Yes
No	Yes	No	No	No	No	No	2	No	No	No	No	No	No	No	No	No	No	No	No
No	Yes	No	Yes	No	No	No	2	yes	No	No	Yes	No	No	No	No	No	No	No	No
No	Yes	No	No	No	No	No	1	No	No	No	No	No	No	No	No	No	No	No	No

Inoquin	Ingenta	Inamin	Innet	Inpip	Intic	Inimi	Inmero	InAug	Inothers	Approp	Cefotaxime	Ceftazidim	Cipro	Genta	Netil
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	Yes	No	No	No	No	No	No	No	yes	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	Yes	No	No	No	No	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	Yes	No	No	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	Yes	No	No	No	No	No	No	No	No	yes	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	Yes	No	Yes	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	yes	Resistant	Resistant	Sensitive	resistant	Sensitive
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	Yes	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	No	Yes	Yes	No	yes	Resistant	Resistant	Resistant	resistant	Resistant

No	No	No	No	No	No	No	Yes	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	Yes	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	Yes	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	Yes	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	Yes	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Sensitive	Sensitive	Sensitive
No	Yes	No	No	No	No	Yes	No	No	No	yes	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	Yes	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Sensitive	Sensitive	Sensitive
Yes	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	Yes	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	Yes	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	Yes	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	Yes	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant

No	Yes	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	Yes	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	Yes	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	Yes	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	Yes	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Sensitive	resistant	Resistant
No	No	No	No	No	No	No	No	Yes	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	Yes	Yes	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	Yes	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Resistant
No	Yes	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	No	Yes	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	Yes	No	no	Resistant	Resistant	Resistant	resistant	Resistant
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	Yes	No	No	No	No	No	No	No	No	no	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	Sensitive	Sensitive
Yes	No	No	No	No	No	No	No	Yes	No	no	Resistant	Resistant	Resistant	Sensitive	Resistant
No	Yes	Yes	No	No	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	Yes	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	No	Yes	No	no	Resistant	Resistant	Resistant	Sensitive	Sensitive
No	No	No	No	Yes	No	No	No	No	Yes	no	Resistant	Resistant	Resistant	resistant	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	Yes	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	resistant	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Resistant	Sensitive	Sensitive

No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	Yes	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	Yes	No	No	Yes	yes	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	No	No	Yes	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	Yes	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Intermedia	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	Yes	yes	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
No	No	No	No	Yes	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	Yes	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	Yes	No	No	Yes	yes	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
No	No	No	No	No	No	No	No	Yes	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	Yes	No	No	No	No	No	No	No	Yes	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Yes	No	No	No	No	No	Yes	No	No	No	yes	Sensitive	Sensitive	Resistant	resistant	Resistant
No	No	No	No	No	No	Yes	No	No	Yes	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Yes	No	No	No	No	No	No	No	Yes	No	yes	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
No	Yes	No	No	No	No	Yes	No	No	Yes	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	Yes	No	No	No	No	No	No	No	No	yes	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	No	no	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
No	No	No	No	No	No	No	No	No	Yes	no	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

Amikacin	Piptaz	Ticarcillin	Cefoperaz	Imipenem	Meropenem	ESBL	PITT's	SAPSII	Predmort	Outcome
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Sensitive	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Intermediate	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Dead
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	2	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	2	.	.	Dead
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Sensitive	Sensitive	Intermediate	Intermediate	Sensitive	Sensitive	yes	0	.	.	Dead
Sensitive	Intermediate	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Sensitive	Resistant	Resistant	Sensitive	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Lost to follow up
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	2	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	4	21	4.2	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	3	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	2	.	.	Alive
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Sensitive	Sensitive	Sensitive	yes	1	.	.	Lost to follow up
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	10	.	.	Dead
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Alive
Sensitive	Intermediate	Intermediate	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	2	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	11	.	.	Dead
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	1	.	.	Alive
Sensitive	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	4	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive

Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	3	.	.	Dead
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	5	58	64	Dead
Sensitive	Intermediate	Resistant	Sensitive	Sensitive	Sensitive	yes	6	62	71.9	Dead
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	1	.	.	Alive
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Sensitive	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Intermediate	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Sensitive	Sensitive	Sensitive	yes	1	.	.	Lost to follow up
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Intermediate	Resistant	Intermediate	Sensitive	Sensitive	yes	4	55	57.5	Alive
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Alive
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Intermediate	Resistant	Sensitive	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	9	.	.	Dead
Resistant	Sensitive	Resistant	Intermediate	Sensitive	Sensitive	yes	3	.	.	Dead
Sensitive	Sensitive	Intermediate	Intermediate	Sensitive	Sensitive	yes	6	.	.	Dead
Sensitive	Sensitive	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	yes	1	.	.	Alive
Sensitive	Sensitive	Resistant	Intermediate	Sensitive	Sensitive	yes	5	59	66.1	Alive
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	6	.	.	Dead
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	4	.	.	Dead
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Sensitive	Intermediate	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Dead
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Alive

Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Intermediate	Sensitive	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Sensitive	Sensitive	Sensitive	yes	7	.	.	Dead
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	0	.	.	Lost to follow up
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Intermedia	Resistant	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	1	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Lost to follow up
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	2	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	3	.	.	Dead
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	1	.	.	Alive
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	9	.	.	Dead
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	3	.	.	Alive
Sensitive	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	4	.	.	Dead
Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	3	25	6.5	Alive
Sensitive	Resistant	Resistant	Sensitive	Sensitive	Sensitive	yes	0	.	.	Alive
Resistant	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	0	.	.	Alive
Sensitive	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	2	.	.	Dead
Sensitive	Intermediate	Resistant	Intermediate	Sensitive	Sensitive	yes	10	.	.	Dead
Sensitive	Resistant	Resistant	Resistant	Sensitive	Sensitive	yes	1	.	.	Dead
Sensitive	Intermediate	Resistant	Sensitive	Sensitive	Sensitive	yes	3	.	.	Alive
Sensitive	Intermediate	Resistant	Resistant	Sensitive	Sensitive	yes	4	.	.	Dead
Sensitive	Resistant	Resistant	Intermediate	Sensitive	Sensitive	yes	8	.	.	Dead
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Dead
Sensitive	Intermediate	Sensitive	Sensitive	Sensitive	Sensitive	No	3	.	.	Alive
Sensitive	Resistant	Sensitive	Intermediate	Sensitive	Sensitive	No	0	.	.	Lost to follow up
Sensitive	Intermediate	Sensitive	Sensitive	Sensitive	Sensitive	No	2	.	.	Alive
Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	No	1	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Lost to follow up
Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	8	.	.	Dead
Sensitive	Intermediate	Intermediate	Intermediate	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	1	34	15.3	Dead
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive

Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	10	54	55.3	Dead
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	2	.	.	Dead
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Lost to follow up
Sensitive	Sensitive	Intermediate	Sensitive	Sensitive	Sensitive	No	1	.	.	Dead
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Intermediate	Resistant	Intermediate	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Resistant	Intermediate	Sensitive	Sensitive	No	1	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	1	.	.	Alive
Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	No	1	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	3	.	.	Alive
Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	No	8	.	.	Dead
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	1	.	.	Alive
Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Resistant	Resistant	Resistant	Sensitive	Sensitive	Sensitive	No	5	.	.	Dead
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Lost to follow up
Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive
Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	No	0	.	.	Alive